

**SOIL FUMIGATION WITH CHLOROBROMOPROPENE  
FOR THE CONTROL OF NUTGRASS<sup>1,2</sup>****BOYSIE E. DAY<sup>3</sup>**

NUTGRASS, *Cyperus rotundus* L., is a perennial sedge which produces tubers (nuts) on creeping rhizomes. The plant is an introduced weed, native to Europe and the tropics, and is believed to have been brought into southern California in the latter part of the nineteenth century. It has become established in irrigated fields and gardens throughout the major agricultural areas of the state. It continues to infest new land, and existing infestations become more firmly established. Because of its extensive rhizome and tuber system, which serves as a means of reproduction and dissemination, it presents a difficult problem in control.

Methods presently available for nutgrass control are expensive in materials or labor and in addition many are of limited effectiveness. Tillage methods are slow and laborious (Davis and Hawkins, 1943; Smith and Mayton, 1939, 1942; Andrews, 1940*a, b*). Smother crops give only partial control (Anonymous, 1938; Holland, 1925; Pollock, 1925; Ranade and Burns, 1925), even when combined with tillage and drying (Davis and Hawkins, 1943). Successful eradication has been reported from flooding for several weeks (Anonymous, 1934; Ranade and Burns, 1925) and from uniform heavy grazing of swine or poultry in small pastures (Mayton, Smith, and King, 1945; Pollock, 1925); but these methods are not always feasible. Of the insects known to attack nutgrass, none appears promising as a means of effective control (Summerville, 1933).

2,4-D and other growth-regulator herbicides have not been generally successful against nutgrass. Applications of 4 or more pounds per acre kill the tops and reduce subsequent regrowth (Burgis, 1949; Burgis and Spencer, 1947; Harrison, 1946), particularly with repeated sprayings (Burgis, 1951). But though the basal bulb and adjacent tubers may be killed, outlying tubers survive (Cowart and Ryker, 1950; Harrison, 1946).

A number of soil sterilants have been tested for the eradication of small infestations. Davis and Hawkins (1943) found that sodium chlorate at rates of 320 to 640 pounds per acre, sodium arsenite at 160 pounds per acre, and

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borax at 1,280 pounds per acre failed to control nutgrass effectively. Success has been reported with sodium chloride and arsenic compounds (Pollock, 1925) and with thiocyanate applied at the rate of 1,400 pounds of  $\text{Ca}(\text{CNS})_2$  simultaneously with tillage (Fromm, 1942). Fumigation has been reported effective with methyl bromide at the rate of 430 pounds per acre when the vapors were retained in the soil by gasproof covers (Leonard and Harris, 1950a, b), with carbon bisulfide at 4,500 pounds per acre (Ciferri, 1931), and with chloropicrin at 400 to 750 pounds per acre (Godfrey, 1939).

Although there is scarcely any limit to the effort or cost one is justified in expending to eliminate small infestations of nutgrass in the early stages of encroachment on fields, the methods used must be highly effective. The present paper reports preliminary experiments with a soil fumigant believed to offer promise for use as a spot treatment in controlling limited infestations of nutgrass.

## LABORATORY EXPERIMENTS

**Methods.** The fumigant used in this study was a commercial formulation of brominated and chlorinated  $\text{C}_3$  hydrocarbons containing about 55 per cent of 1-chloro-3-bromopropene (CBP).<sup>4</sup> This material is used as a soil fumigant for the control of nematodes, wireworms, and soil-borne fungi.

Laboratory experiments on the toxicity of CBP to nutgrass as affected by concentration, temperature, and time were done by variations of the following standard procedure, used in this laboratory for investigating the response of nutgrass to a wide variety of halogenated hydrocarbons.

Nutgrass tubers were collected from the field, attached roots and rhizomes were removed, and uniform tubers weighing about 1 gram were selected. Tubers damaged by insects or injured in the process of digging were discarded.

Each toxicity test was made by placing 50 tubers in a 500-ml Erlenmeyer flask and adding 30 grams (about 250 ml) of horticultural vermiculite. The fumigant was added in liquid form (usually 0.01 to 0.05 ml) by means of a microburette. The flask was quickly stoppered and the contents were shaken to distribute the tubers throughout the vermiculite. With the exception of experiments in which temperature effects were determined, the flasks were allowed to stand at room temperature (23° to 25° C) during fumigation. The tubers were then removed, washed in water, and aerated for 1 hour.

Germination tests were made immediately after aeration. Tubers were planted on thick blotting paper in 10 by 11 cm plastic trays and placed in a moist germinator at 30° C for 7 days. The cultures were examined and sprouted tubers, having either roots or shoots, were counted. Those not sprouting were found to be discolored internally and to have undergone tissue breakdown; this was indicated by a leaching of brown material, which stained the blotters.

In some tests the flasks were completely filled with soil instead of vermiculite. The soil was air-dry (0.23 per cent moisture) Ramona sandy loam screened free of gravel. The fumigant was injected into the soil at about the center of the flask. Otherwise the procedure was the same as for the vermiculite tests.

<sup>4</sup> Formula CBP-55, supplied by Shell Chemical Corporation, San Francisco, California.



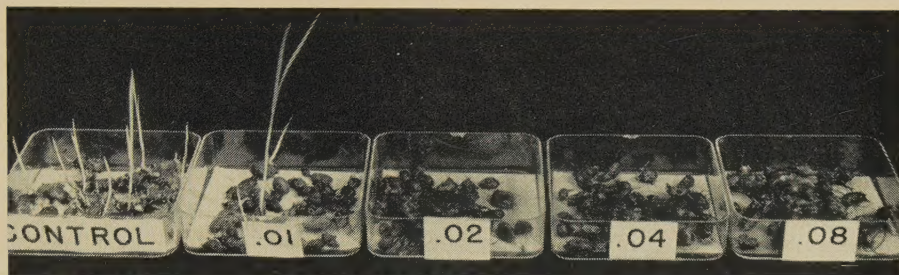


Fig. 1. A determination of the toxicity of the fumigant. Tubers were mixed with vermiculite in 500 ml flasks and fumigated for 18 hours with .01, .02, .04, and .08 ml of CBP formulation. Sprouting tests were then made. The control was not fumigated.

**Dosage in Vermiculite.** When 0.00, 0.01, 0.02, 0.04, and 0.08 ml of CBP formulation was added to test flasks containing 50 tubers and 30 grams of vermiculite in the usual manner and fumigated for 18 hours, 41 tubers of the control survived, 5 tubers of the 0.01 ml treatment survived, and all others were killed (fig. 1). The 0.02 ml dosage, which gave a complete kill, is equivalent to 0.04 ml per liter of confined space.

**Time of Fumigation.** Tubers were fumigated by treatments of 0.00, 0.01, 0.02, and 0.03 ml of CBP formulation for 1, 3, 5, 8, 12, 16, 20, 24, and 30 hours. Data are shown in Figure 2. The kill was close to maximum in about 5 hours.

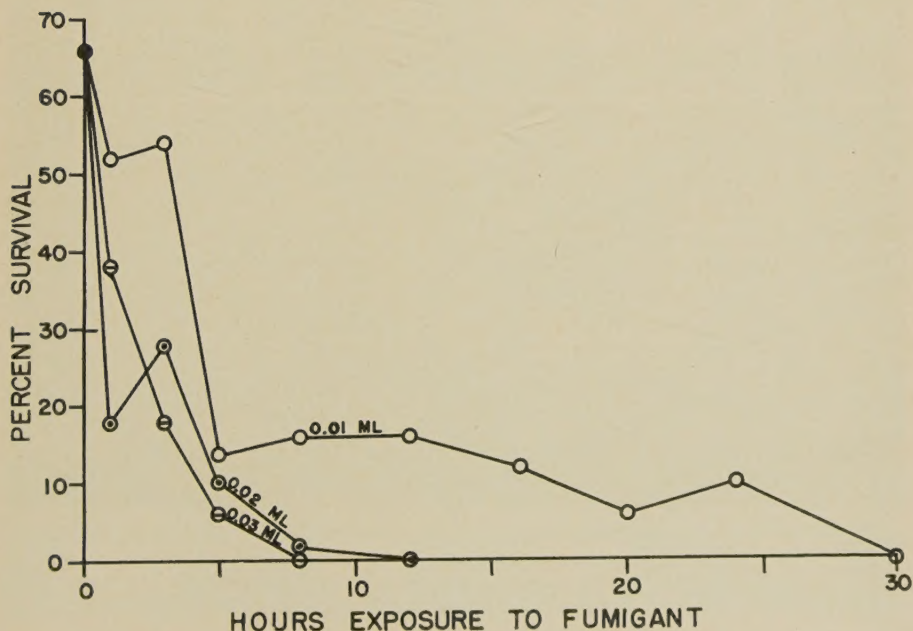


Fig. 2. The effect of length of time of fumigation with different amounts of CBP formulation on survival of tubers.

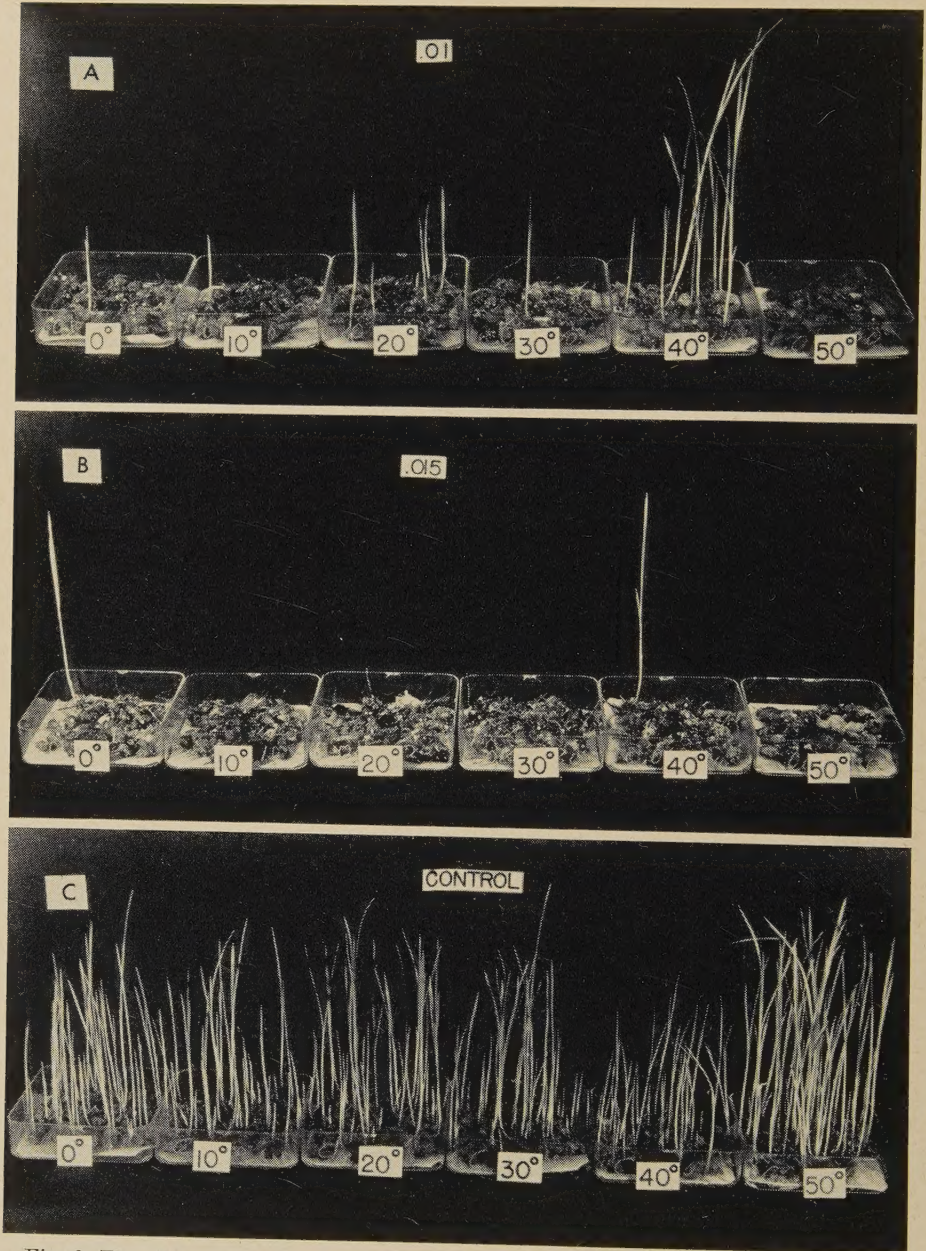


Fig. 3. The effect of temperature during fumigation on the survival of nutgrass tubers treated with: A, .010 ml of fumigant per 500 ml fumigation chamber. B, .015 ml. C, control (not fumigated). Toxicity of the CBP formulation is largely independent of temperature.



TABLE 1  
PERCENTAGE OF NUTGRASS TUBERS SURVIVING CBP FUMIGATION  
AT SIX TEMPERATURES

Samples of 50 Tubers Fumigated 18 Hours in 500-ml Flasks of Vermiculite

Amount of CBP formulation, ml per flask	Percentage of tubers surviving fumigation					
	At 0° C	At 10° C	At 20° C	At 30° C	At 40° C	At 50° C
0 (Control).....	96	86	90	94	90	90
0.10.....	2	2	14	2	18	0
0.15.....	4	0	0	0	2	0

Survival at longer exposures was not closely correlated with time of fumigation, and in some tests was less than at 5 hours.

**Temperature.** Groups of 50 tubers were fumigated in vermiculite for 18 hours at temperatures of 0°, 10°, 20°, 30°, 40°, and 50° C. At each temperature samples of tubers were treated with 0.00, 0.10, and 0.15 ml of CBP formulation. The results are shown in Table 1 and Figure 3, a photograph

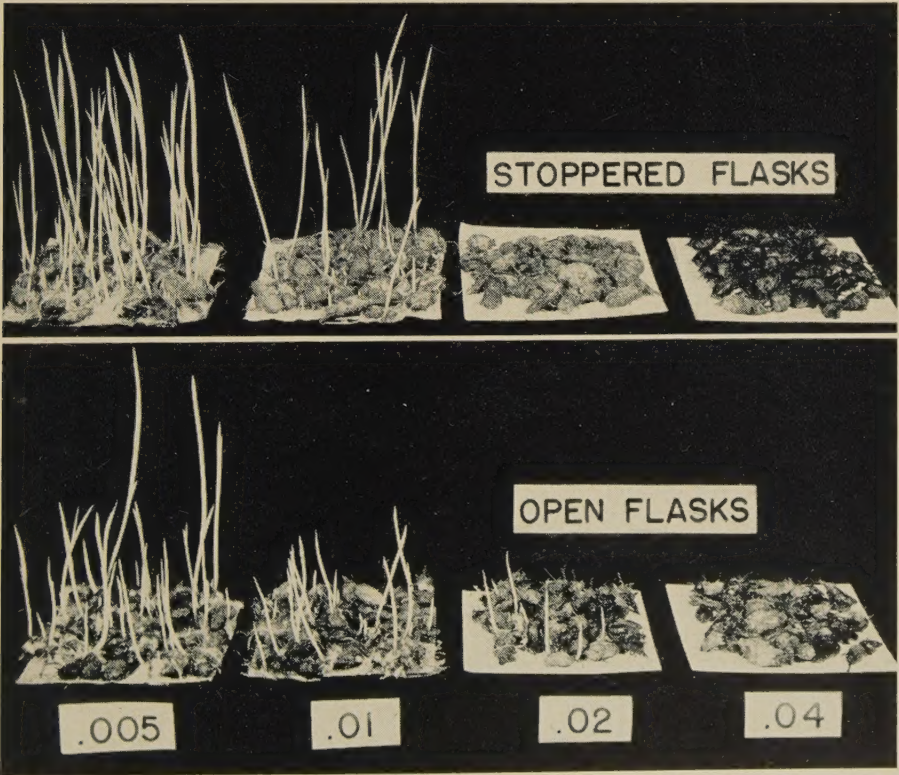


Fig. 4. Retention of CBP vapors in toxic concentrations by soil. Tubers were mixed with soil, placed in 500 ml flasks and fumigated with 0.005, 0.01, 0.02, and 0.04 ml of CBP formulation. Survival of tubers was determined in stoppered flasks (above) as compared with open flasks.

taken at the end of the germination period. Temperature appears to have little influence on the lethal action of the fumigant.

**Dosage and Persistence in Soil.** To determine the influence of the presence of soil on toxicity and to determine the persistence of CBP in toxic quantities in soils, tests were made with soil in place of vermiculite. Two series of fumigations were carried out, using 0.005, 0.01, 0.02, 0.04, and 0.08 ml of CBP formulation per flask. The tubers were mixed with the soil in the flasks, after which the soil was injected. In one series the flasks were stoppered, in the other left open. In both, the tubers were fumigated for 18 hours. Results are

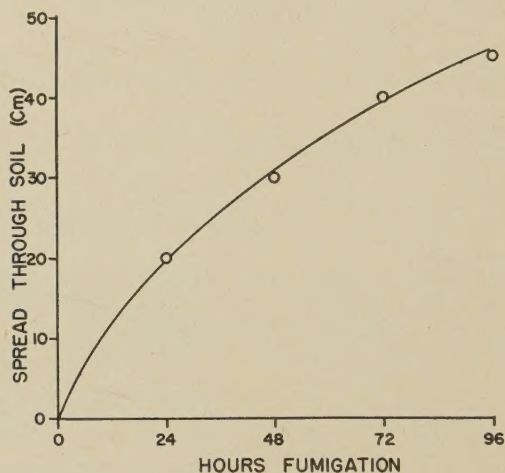


Fig. 5. Fumigant was allowed to diffuse from a saturated vapor through a column of soil containing nutgrass tubers. The extent of spread of the fumigant after various lengths of time is shown.

shown in Figure 4. No tubers survived dosages of 0.04 or higher. Seven tubers—14 per cent—survived in the open containers at 0.02 ml of CBP formulation; however, this experiment demonstrates that even so shallow a depth of soil as that in the flasks will retain CBP vapors long enough to be lethal to nutgrass tubers.

**Rate of Spread.** Preliminary to field trials, the rate of spread of the lethal effects of CBP formulation through soil was determined.

A cotton plug was placed in the bottom of a 1-meter length of 3-cm glass tubing held vertically on a table, and 10 cm of Ramona sandy loam soil was tamped into the tube. Ten nutgrass tubers were dropped into the tube, and 5 cm of soil was packed upon them. More tubers were added, followed by more soil, and this procedure was repeated until 12 groups of tubers had been packed at 5-cm intervals along the tube. A plug of absorbent cotton was placed on top of the uppermost layer of soil, and this was moistened with 1 ml of CBP formulation to provide a surplus of CBP to maintain a saturated vapor. The upper end of the tube was stoppered and the bottom allowed to remain open.



Four such tubes were prepared, with the CBP formulation added to one of the tubes every 24 hours. After tubes had been fumigated for 24, 48, 72, and 96 hours respectively, all were opened, and viability of the tubers was determined in germinators in the usual way.

The distance to which a lethal amount (100 per cent kill) of fumigant diffused ranged from 20 cm in the 24-hour treatment to 45 cm in the 96-hour treatment (fig. 5).

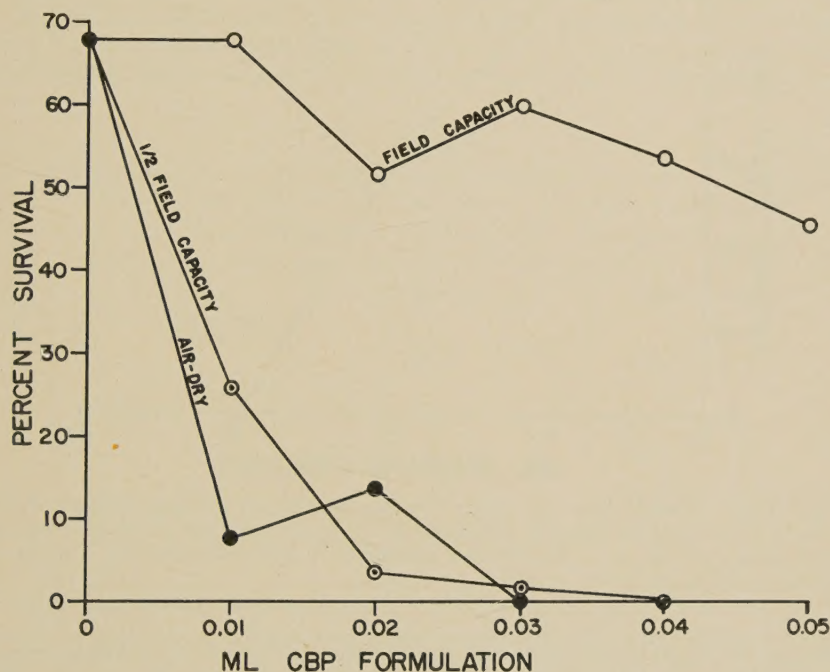


Fig. 6. Germination of tubers after fumigation in soils of three moisture levels contained in 500 ml flasks. Varying amounts of fumigant were injected into the center of the soil mass. Soil at field capacity protected the tubers from high concentrations of CBP.

**Soil Moisture.** The effect of soil moisture on the action of the fumigant was determined. Fifty nutgrass tubers were mixed with 250 ml of soil containing the following amounts of moisture: 21.4 per cent (field capacity), 10.7 per cent, and 0.23 per cent (air-dry). The soil-tuber samples were placed in 500 ml flasks. A sample of each soil moisture content was fumigated with 0.01, 0.02, 0.03, 0.04, and 0.05 ml of CBP formulation injected near the center of the soil mass. Flasks were stoppered for 18 hours, after which the nutlets were removed, washed, aerated, and placed in germinators in the usual manner. Results are shown in Figure 6.

There was little difference between survival in dry soil and in soil at one half of field capacity; however, soil at field capacity afforded a high degree of protection against the fumigant. This protection could be due to an interaction between the soil moisture and the active ingredient of the fumigant

or to a blocking of the pore space of the soil. The fact that increasing the concentration of fumigant did not overcome the protective action of soil moisture indicates that the paths for gaseous diffusion had been blocked. This observation is in agreement with Hagan's (1941) finding that the permeability of soil to  $\text{CS}_2$  vapor approaches zero not at zero porosity but in a porosity range of 26 to 29 per cent.

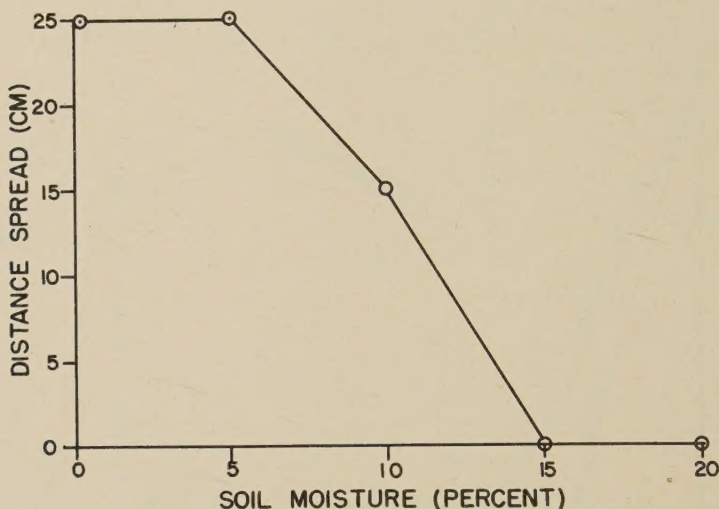


Fig. 7. Effect of soil moisture on distance of movement of soil fumigant during a 48-hour period. Fumigant was permitted to diffuse for 48 hours through soils of different moisture content having groups of nutgrass tubers embedded at 5-cm intervals. The distance the fumigant moved is shown.

The effect of soil moisture on diffusion of the fumigant was further investigated by the method used in determining rate of spread. Five glass tubes were filled with soil containing respectively 0.23 (air-dry), 5, 10, 15, and 20 per cent moisture. Samples of 10 tubers were spaced at 5-cm intervals and fumigant was applied as before. After 48 hours' exposure to the fumigant, tubers were removed and their viability determined. A complete kill of all tubers was attained for 25-cm distance in both the air-dry soil and soil containing 5 per cent moisture (fig. 7). The lethal effect of the fumigant spread 15 cm through soil containing 10 per cent moisture and less than 5 cm in soils having 15 and 20 per cent moisture.

Reduction of the porosity of the soil by adding water and by handling it while moist (puddling) accounts for the lack of movement of the fumigant at higher moisture contents. The movement of toxic quantities of CBP is limited by the same factors as that of carbon disulfide (Hagan, 1941; Hannesson, 1945; Hannesson, Raynor, and Crafts, 1945).



## FIELD EXPERIMENTS

**Methods.** Field experiments were performed near Upland, California, during September and October, 1951. The soil was Hanford fine sandy loam. The portion of the field that was used in tests was covered with a uniform stand of young nutgrass 4 to 8 inches high and having about 6 shoots per square foot. Seedstalks were not present. The soil contained an average of 70 tubers per square foot in the surface 6 inches and 35 per square foot at the 6 to 12 inch depth, as determined by screening out six 2-square-foot plots. No tubers were found at depths lower than 12 inches in the areas used, although tubers have been found as deep as 22 inches in the same field on other occasions.

TABLE 2  
PERCENTAGE OF NUTGRASS TUBERS SURVIVING FIELD TREATMENT WITH CBP FORMULATION AT DIFFERENT RATES  
Fumigant Injected to 8-inch Depth with 12-inch Spacing

Treatment rate, gallons per acre	In 0-6 inch depth		In 6-12 inch depth	
	Total tubers	Per cent of tubers surviving	Total tubers	Per cent of tubers surviving
0 (Control).....	113	20	34	35
10.....	344	16	161	50
20.....	124	20	53	32
40.....	61	12	34	0
80.....	46	7	34	3
160.....	70	0	36	0

CBP formulation was injected 8 inches deep with a Mack weed gun at varying rates and spacings in tilled and untilled soil under several moisture conditions. Plots were 25 or 100 square feet. One control plot was used with each experiment.

**Field Dosage Test.** For tests of dosage under field conditions, an area 10 feet wide and 60 feet long was divided into six equal squares. The area had been disked to a depth of 6 inches 6 weeks earlier and at that time two irrigation furrows about 4 inches deep had been laid out, running the length of the area. Irrigation water had been run down the furrows shortly after the disk-ing; it settled the soil in the furrows and caused some crusting and compaction of the soil around the furrows. The soil below 6 inches in depth was compact.

CBP formulation was applied at rates of 0, 10, 20, 40, 80, and 160 gallons per acre. Injections were made on a square pattern of 12-inch spacing. In making applications at the lower rates (10 to 80 gallons per acre) it was found convenient to dilute the CBP formulation with cleaning solvent (naphtha) to secure sufficient volume for effective functioning of the applicator. Tests of the diluent alone failed to indicate measurable toxicity in the amounts used.

Five days after the fumigant was applied, the soil of a 2-square-foot area at the center of each plot was screened, and all tubers were placed in germi-

nators. The per cent of viable tubers was determined for the top 6 inches and for the 6 to 12 inch depth.

Aerial growth of nutgrass was killed on all treated plots in the first 2 days after injection of the fumigant. However, living tubers were found in the soil in all plots except the one treated at the 160-gallon rate (table 2). In the 2 months after treatment there was extensive regrowth on the plots treated with 10 and 20 gallons. Regrowth after the 40-gallon treatment was sparse with the exception of the compacted furrows, where luxuriant growth occurred. Growth on the 80-gallon plot was limited to a sparse stand in the irrigated furrows. No regrowth followed the 160-gallon treatment.

TABLE 3  
PERCENTAGE OF NUTGRASS TUBERS SURVIVING FIELD TREATMENT WITH CBP FORMULATION IN UNTILLED SOIL CONTAINING VARYING AMOUNTS OF MOISTURE  
Fumigant Injected to 8-inch Depth at 12-inch Spacings; Applied at the Rate of 80 Gallons per Acre

Per cent soil moisture	In 0-6 inch depth		In 6-12 inch depth	
	Total tubers	Per cent of tubers surviving	Total tubers	Per cent of tubers surviving
2.05.....	91	7	35	0
3.19.....	193	10	39	0
3.27.....	74	20	12	8
5.62.....	96	7	132	0
7.22.....	68	16	15	7

**Untilled Soil with Varying Soil Moisture.** Another experiment in the same field was planned to take advantage of a variation in soil moisture without apparent difference in nutgrass population. Fumigations at the rate of 80 gallons per acre, with 12-inch injection spacings, were made to five plots each 5 feet square in an area of firm, untilled soil near an irrigation stand-pipe. Soil-moisture determinations made at the time of fumigation showed that the moisture varied from 2.0 to 7.2 per cent on the different plots. The field capacity of the soil was not determined. The compact untilled soil offered a further comparison with similar experiments, made on the same date, in well-tilled soil (see the next section).

Kill was incomplete in all plots without apparent correlation with soil moisture (table 3). A higher kill was attained in the layer 6 to 12 inches below the surface than in the upper 6 inches of soil.

**Tilled Soil.** In still another area in the same field the soil was loose and open to a depth of 6 inches as a result of recent tillage. At lower depths the soil was compact but without a pronounced plowsole. Four adjacent 25-square-foot plots in this area were fumigated with CBP formulation at the rate of 80 gallons per acre in a square pattern of injections spaced 6, 8, 10, and 12 inches respectively. A control plot was not fumigated.

Two weeks after the fumigant was applied, tubers were screened out of 2 square feet of soil of each plot to a depth of 12 inches. Ninety-five tubers



were recovered from the control, of which 39 were viable. The treated plots yielded an average of 108 tubers per sample, none of which had survived the fumigation.

These plots, located in the same field as those in the preceding experiment, afford an opportunity for comparing the effectiveness of the fumigant in an open, well-tilled soil and in a compact, untilled soil. Presumably the recent tillage accounts for the effectiveness of the fumigant under all conditions of injection spacings in the present experiment. CBP movement is severely restricted by soil compaction and resultant reduction of porosity in the same way as carbon bisulfide (Hagan, 1941; Hanneson, 1945; Hanneson, Raynor, and Crafts, 1945).

## DISCUSSION

The concentration required for an assured kill of tubers under laboratory conditions is on the order of 0.04 ml of CBP formulation per liter of confined space (0.02 ml per test) in fumigation flasks containing vermiculite, and approximately the same amount per liter of soil. Fumigation for prolonged periods is not required: maximum kill was obtained with 5 hours' fumigation. The effectiveness of the fumigant was largely independent of temperature but was reduced when soil moisture was at field capacity. Tubers are quickly killed without induction of dormancy.

On the basis of laboratory experiments, it is estimated that fumigation with about 13 gallons per acre-foot should be required for effective control of nutgrass under ideal conditions in the field. For fumigation to a depth of 2 feet a basic requirement of about 26 gallons must be applied plus an additional amount to compensate for wastage, soil compaction, and other deviations from ideal conditions.

Although exact doses for practical use have yet to be determined under field conditions, 80 gallons per acre proved to be effective with injection spacings of 6 to 12 inches on moderately dry, well-tilled soil; 160 gallons per acre was effective in a light soil tilled and irrigated 6 weeks before fumigation.

CBP promises to provide an effective means of spot treatment of small infestations of nutgrass. It gives an immediate kill of all vegetative parts and may be conveniently applied without the use of tarpaulins or other special equipment.

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**EFFECT OF 2,4-D UPON THE DEVELOPMENT  
OF THE COTTON LEAF**

**ERNEST M. GIFFORD, JR.**



Fig. 1. Comparison between a young cotton plant treated with 15 gamma 2,4-D (right) and an untreated cotton plant (left). Note precocious development of axillary buds on the treated plant.

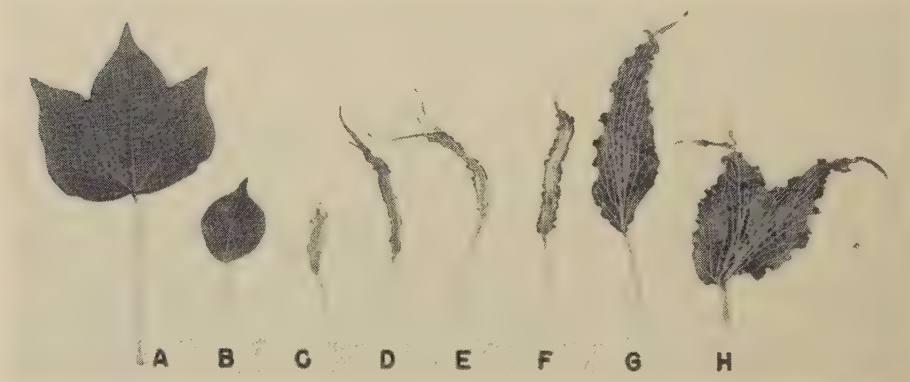


Fig. 2. A selected series of cotton foliage leaves showing degrees of symptomatic response to treatment with 2,4-D. *A*, Normal leaf; *B-H*, the first seven leaves of a treated plant shown in order of appearance at the shoot apex. Note the crowding of veins and restriction of photosynthetic tissue to the margins in *D-F*. Recovery is evident in *G-H*. The leaf shown in *H* represents a fusion of two leaves.



# EFFECT OF 2,4-D UPON THE DEVELOPMENT OF THE COTTON LEAF<sup>1</sup>

ERNEST M. GIFFORD, JR.<sup>2</sup>

## INTRODUCTION

A SERIES of histological studies was undertaken in 1950 to learn more about how 2,4-dichlorophenoxyacetic acid (2,4-D) affects the vegetative growth of cotton, *Gossypium hirsutum* L. var. Acala, 4-42. Efforts were directed chiefly at determining (1) whether 2,4-D alters the shoot apical meristem or whether it affects the development of foliage leaves only after their initiation from a morphologically unaltered shoot apex; (2) how long its effects persist—that is, whether it affects leaf primordia not yet initiated in the bud at the time of treatment; (3) how it affects the development of the lamina; and (4) how it affects early vascularization.

In the decade preceding the announcement of 2,4-D, many papers appeared dealing with both the gross morphological and the detailed histological responses of plant tissues to a wide variety of hormones and substances that were hormonelike in their effects. Thomson (1945) gives a helpful review of these papers.

Since the discovery that application of 2,4-D to the foliage of certain broad-leaved plants results in death, extensive research has been carried on to determine the nature of the toxic action. The research has dealt not only with the purely herbicidal potentialities of this hormonelike substance, but also with the morphological and histological changes that take place under different environmental conditions and with various concentrations of the chemical. Reference will be made, here and later, only to those studies that seem most pertinent to the present investigation.

Any theory that attempts to explain the action of 2,4-D on plant tissues must account for observed structural changes in the plant. In cotton this herbicide induces modifications in both floral and vegetative parts similar to those it or other physiologically active substances induce in tomatoes (Zimmerman and Hitchcock, 1941) and beans (Burton, 1947; Felber, 1948; Eames, 1951). The most apparent aberration is the bizarre shape and lighter color often assumed by the foliage leaves. Severely affected leaves or leaflets become straplike (fig. 1, right), with closely apposed veins (fig. 2). In extreme cases the margins of the leaf become convoluted and contain most of the photosynthetic tissue. Often irregular patches of chlorenchyma may occur between the veins. Recovery of the treated plant can be expected if the dosages have not been severe.

In order to understand the histological alterations in cotton leaves caused by 2,4-D applications, one must have a clear picture of their normal ontogeny. General organography of the cotton shoot in three varieties of cotton has been described by Gore (1935). He has presented three-dimensional drawings depicting the organogeny of reproductive shoots and a few details of vegeta-

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tive development. Only one diagram deals with the details of cellular arrangement in the shoot apical meristem and in a young leaf primordium. Burton (1950) has described the ontogeny of the normal cotton leaf and compared it briefly with that of the plant treated with 2,4-D. The present study is intended to augment Burton's study on normal cotton and to present a more complete description of the development of the modified leaf.

## MATERIALS AND METHODS

Seeds of cotton were germinated in soil in the greenhouse. When the cotyledons were expanded fully each plant was treated with 15 gamma of the acid of 2,4-D by dropping this amount (from a 500 p.p.m. solution) on the surface of the cotyledons with a special micropipette. Preliminary experiments had established this dosage as the amount of 2,4-D which would produce maximum external symptoms and yet permit the recovery of the plants. With this method, a surprisingly uniform response on the part of all plants occurred. Some plants were left untreated to provide the necessary controls.

Plants were treated on April 2, 1950, and weekly collections made through July 14, 1950, at which time many of the remaining plants had recovered completely. Shoot material was killed in chromic acid-propionic acid-formalin mixtures, dehydrated following an ethyl alcohol-xylene schedule in which the highest concentration of alcohol used was 96 per cent. Alcohol of this percentage is quite miscible with xylene. Material processed in this way does not become excessively hard and brittle and can be sectioned successfully. All material was embedded in Tissuemat, sectioned at 6-10 microns and stained with tannic acid-iron chloride-safranin (Foster, 1934).

## FORMATIVE EFFECTS AND PERSISTENCE OF 2,4-D

As has been shown in other studies, the severity of leaf distortion is correlated with the stage of differentiation of any particular leaf at the time of treatment. Although no foliage leaves had emerged from the bud when the plants were treated, the first foliage leaf can be considered to have been in an advanced stage of development; and it showed comparatively little modification (fig. 2). Successive leaves showed an increase in deformation, then a decrease; complete recovery is generally achieved. Treated plants showed considerable range in the number of leaves affected: the leaf most severely distorted varied from the second to the fifth, and the number of leaves showing some symptoms ranged from five to ten.

Several seedling plants were sampled at the cotyledon stage to determine how many leaves had been initiated at the time of treatment. None were found that had more than six leaves or leaf primordia. Yet in most treated plants more than six leaves showed symptoms. Thus it appears that 2,4-D is translocated to the shoot apex and continues to affect leaf primordia not present in the bud when the application was made.

## EFFECT ON THE SHOOT APICAL MERISTEM

Cellular organization within the apical meristem of dicotyledonous shoots can be interpreted as consisting of two growth zones distinguished from each



other by different planes of cell division. The tunica consists of one or more layers of cells which divide predominately in the anticlinal plane, that is, a new cell wall is formed perpendicular to the surface of the shoot apex. If there is more than one layer in the tunica, periclinal divisions may occur in



Fig. 3. Longitudinal section through the shoot tip of an untreated cotton plant showing general organization of that portion of the plant body. ( $\times 60$ .)

the cells of the innermost layer. Internal to the tunica is a group of cells, termed the corpus, in which planes of cell division are variable. Moreover, apical meristems in angiosperms may show degrees of cytohistological differentiation within the tunica and corpus as described below.

The shoot apex of cotton (var. Mebane) described and illustrated by Gore (1935) could be interpreted as having a two- or three-layered tunica even though the terms tunica and corpus were not employed by that author. In the present study, the apical meristem of the untreated plant (figs. 3 and 4, *A*) consists of a two-layered tunica (here referred to as  $T_1$  and  $T_2$ ) in which the discreteness of the second tunica layer is often obscured by periclinal or oblique divisions, particularly along the flanks of the shoot apex. Generally the corpus initials are enlarged slightly, apparently quite vacuolate, and

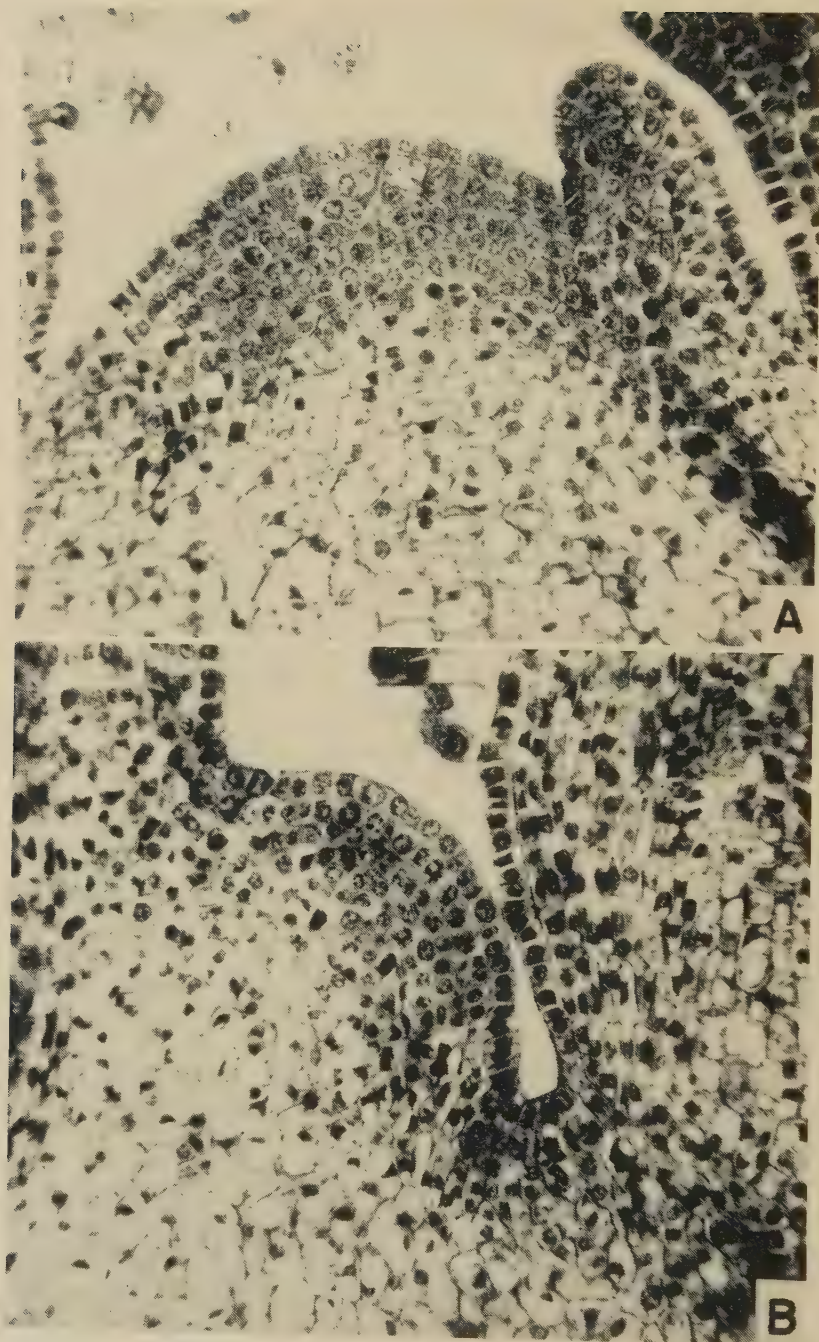


Fig. 4. Median longitudinal views of the shoot apices of an untreated cotton plant (A) and a cotton plant treated with 2,4-D (B). ( $\times 300$ .)



stain much more lightly than their immediate derivatives (fig. 4, A). Relatively large cell size and light staining are often observable in the tunica layers as well, particularly in  $T_2$ . Such differences in cellular morphology between the cells in the zone of initials and those derived from the initials are not unique in cotton; their occurrence has been reported in many genera (Philipson, 1949; Gifford, 1950; Popham, 1951).



Fig. 5. Outline diagrams of developing foliage leaves from an untreated cotton plant. The primordia shown in face view are seen from the adaxial side (side toward the stem axis). Representative transverse sections of primordia L2 and L3 are shown above or to one side of the particular leaf. See text for complete explanation. Height of primordia in microns: L1, 80; L2, 488; L3, 1016.

On a purely structural basis there are no significant differences between the apices of untreated and treated plants at the dosage used. In treated plants (fig. 4, B) the apex often appears to be turned or deflected slightly to one side. This can be accounted for, possibly, by the altered type of leaf development described below. There is no significant difference in size, although dimensions of width during the maximal-area phase (that is, during enlargement of the shoot apex prior to the emergence of a leaf) tend to be greater in the control plants (171 microns wide and 46 microns high). In treated plants the corresponding average dimensions of the same plastochron stage are 150 by 56 microns. The measurements were taken at the base of the youngest foliage leaf primordium and included some examples in which early divisions, leading to the formation of the next foliage-leaf primordium, were in evidence.

In some studies by other workers, measurements of maximal-area phase include only that portion of the shoot apex above definite leaf primordia.

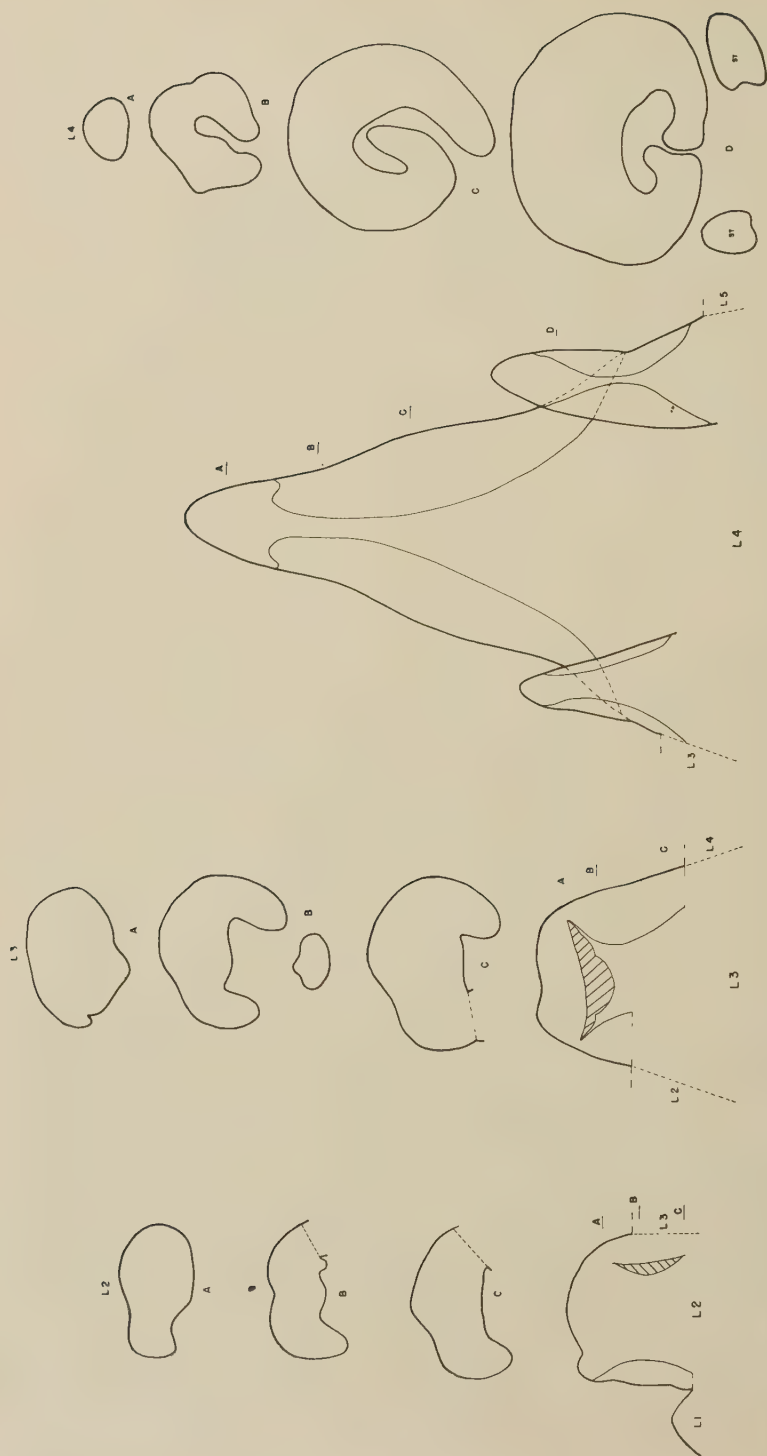


Fig. 6. Outline diagrams of developing foliage leaves from a cotton plant treated with 2,4-D. The extent of leaf fusions is indicated by a broken line along the lower flanks of the primordia. The area indicated by hatching in *L2* is an "enclosed" stipular flange. In *L3* the tip of the leaf is recurved, the free portion indicated by hatching. Transverse sections, corresponding to levels indicated on the primordia as seen in face view, are shown above or (for *L4*) to one side of the particular developmental stage. Height of primordia in microns: *L2*, 530; *L3*, 640; *L4*, 1,575.



## FOLIAGE-LEAF DEVELOPMENT IN TREATED AND UNTREATED PLANTS

**General.** Several stages in the development of foliage leaves are shown diagrammatically in Figure 5 for untreated plants and in Figure 6 for treated plants. These diagrams have been constructed to scale from a series of consecutive transverse sections.

In untreated plants, a young primordium is quite broad from the onset of leaf development (fig. 5, *L1*). Very early the leaf tip becomes oblong, stipules are formed on the lower flanks, and the tips of the lateral leaf lobes become apparent as a result of differential lateral growth (fig. 5, *L2*). Stipules elongate precociously. The median and lateral leaf lobes of the future foliage leaf form lateral flaps of tissue, which collectively will become the leaf blade, or lamina. The entire leaf and stipule complex becomes a five-fingered growth unit (fig. 5, *L3*).

In the treated plant both radial and lateral growth of primordia are uninhibited. The four young primordia of this shoot are fused laterally through a portion of their bases. (Lines of fusion are indicated by the dotted lines in the diagrams *L2*, *L3*, and *L4* in fig. 6). In extreme injury, certain primordia may be deformed very early, the terminal growing portion forming a sheath around the next younger primordium (fig. 6, *L3*). In severely affected plants, stipule initiation is delayed, and the stipules do not become free from the leaf for some distance. When a tubular sheath is formed, stipules may be represented only as uniseriate flaps arising from the adaxial side of the developing primordium (fig. 6, *L2*). A characteristic feature is the delayed appearance of definite lateral leaf lobes (fig. 6, *L4*).

**Leaf Initiation.** During the maximal-area phase in both treated and untreated plants, the shoot apex becomes asymmetrical, the enlarged flank constituting the foliar buttress. As in many dicotyledons (Gifford, 1951), the periclinal divisions which definitely determine the locus of leaf inception occur in the second layer of the apical meristem—in cotton, the second tunica layer (fig. 7, *A*). After these divisions, or often concomitantly with them, periclinal divisions take place in the derivatives of the corpus initials and give rise to cells which eventually become part of the leaf base.

At the first appearance of periclinal divisions in the second tunica layer of an untreated plant, a procambial strand is established at the base of the new leaf in continuity with procambium present below in the axis (fig. 7, *A-C*). The cells of this strand show the usual cytological characteristics of procambium: they have relatively denser cytoplasm and chromaticity than cells of the future pith and cortex.

**Early Stages in Leaf Development.** While leaf initiation appears to be the same in treated and untreated plants, differences occur in early stages.

In untreated plants the leaf primordium shortly after initiation undergoes a period of apical growth by active cell division of a subapical initial or initials. This type of growth continues until the primordium is approximately 1,200 microns high. There is early vacuolation of cells on the abaxial and adaxial sides of the primordium, in a pattern common to many dicotyledons

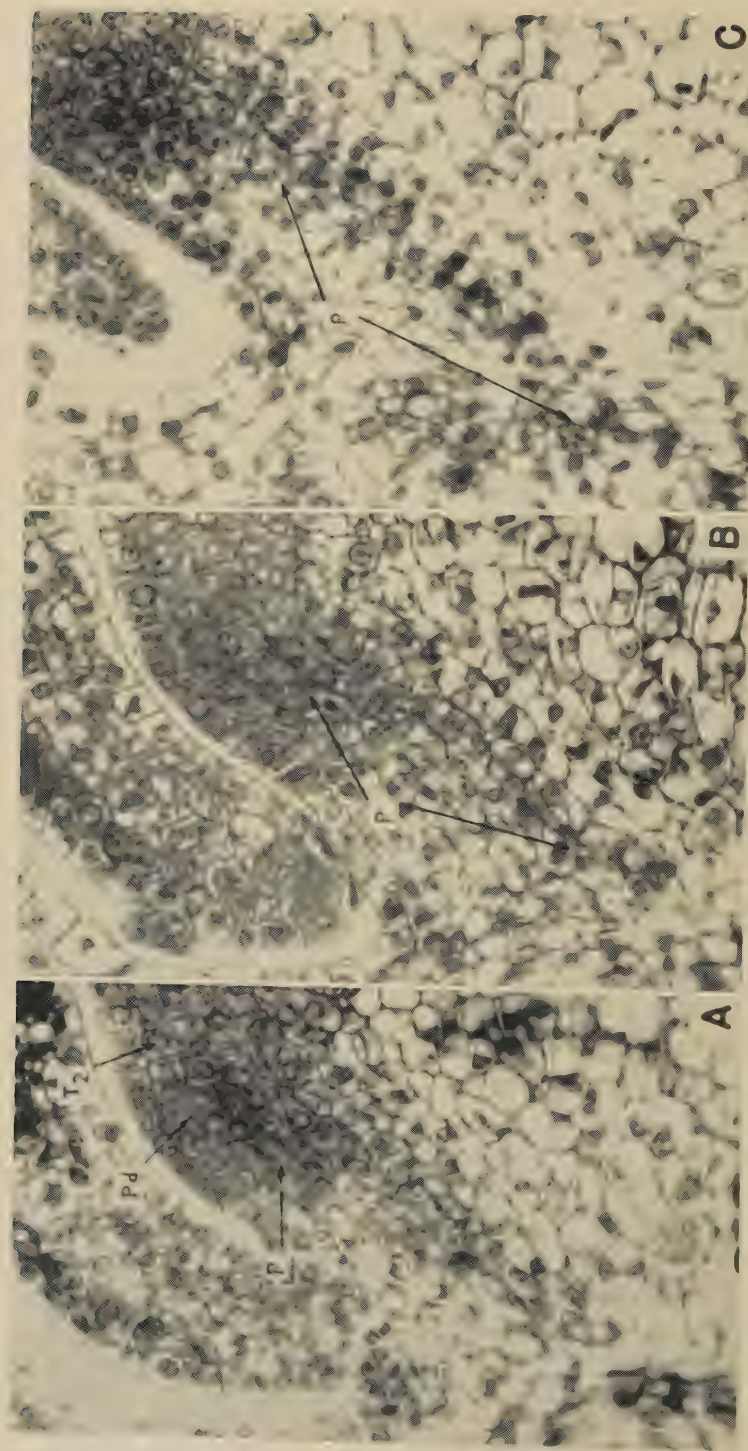


Fig. 7. Adjacent longitudinal sections of a shoot apex showing early establishment of procambium in relation to the initiation of a foliage-leaf primordium in an untreated cotton plant. *P*, Procambium; *Pd*, results of a periclinal division and subsequent anticlinal divisions; *T<sub>2</sub>*, second anticlinal layer. ( $\times 4$ .)



(Gifford, 1951). Then a generalized type of intercalary growth occurs, rapidly increasing the length of the primordium. When the primordium is approximately 200 microns high, an adaxial meristem becomes functional near the base of the primordium and produces a definable bulge as the leaf grows.

In treated plants a leaf primordium after initiation undergoes rapid lateral and radial extension (fig. 6, *L2*, *L3*). Instead of forming a steep cone as in normal plants, young primordia are often flat to bluntly conical at the distal end (fig. 6, *L2*, *L3*); as mentioned earlier, the tip of the primordium is sometimes turned back upon itself, overarched younger primordia (fig. 6, *L3*). An adaxial meristem becomes functional when the primordium is 200 to 300 microns high—about the same as in untreated plants—but the prolonged continuation of this activity in treated plants plays an important role in determining the final leaf structure.

The production of leaves in treated plants (at the dosage used) continues at a rate similar to that in untreated plants, but internodal elongation is much reduced; hence a leaf produced during one plastochron is not separated much in the longitudinal direction from the leaf of the next plastochron. As a result, in severely affected plants a new leaf becomes fused laterally with the next older leaf (fig. 6). Fusions are generally restricted to the leaf bases, but sometimes involve a considerable portion of each leaf. The joined leaves form a cylinder around the shortened axis, and the entire composite structure grows as a single unit. McIlrath, Ergle, and Dunlap (1951) have described the encasement in a foliar tube of the terminal bud of a cotton seedling produced from seed pretreated with 2,4-D while still in the boll.

**Marginal Growth of Untreated Leaves.** After the establishment of an original axis (the future petiole and midrib of the foliage leaf) the upper portion of this axis becomes extended laterally through marginal growth, resulting in the formation of the future lamina. The base of the axis, which will become the future petiole, increases in breadth by a generalized type of marginal growth—that is, by periclinal divisions in cells located beneath the protoderm. When a primordium is approximately 120 microns high, definite submarginal initials become apparent on the sides of the primordium, beneath the protoderm, in that portion which will give rise to the blade.

There is considerable fluctuation in the mode of marginal growth during early laminal formation. At the onset of marginal growth, whether it be the first appearance of marginal activity in a young primordium or a continuation of such activity near the tip of an older primordium, a certain amount of fluctuation occurs in the sequence of divisions in submarginal initials and their immediate derivatives. A submarginal initial may divide in a rhythmic fashion giving rise to an abaxial layer and an adaxial layer. The cells of these lineages may then undergo periclinal divisions almost immediately. In this way a developing ridge of four layers is established (figs. 8, *A*, and 9, *A*). Often the submarginal initial undergoes divisions giving rise to an abaxial layer, an adaxial layer, and a middle layer. The developing mesophyll is then increased to four layers by periclinal divisions in the abaxial layer (fig. 8, *C*) or frequently by similar divisions in the adaxial layer or less frequently by divisions in the middle layer (fig. 8, *B*, *PD*), not associated with vascular bundle formation.

Once marginal growth is initiated by the methods outlined above, a rather uniform type of growth takes place. The submarginal initials continue to give rise to the three layers: the adaxial layer, the abaxial layer, and the middle layer (fig. 9, *B-D*). The adaxial layer, perpetuated by anticlinal di-

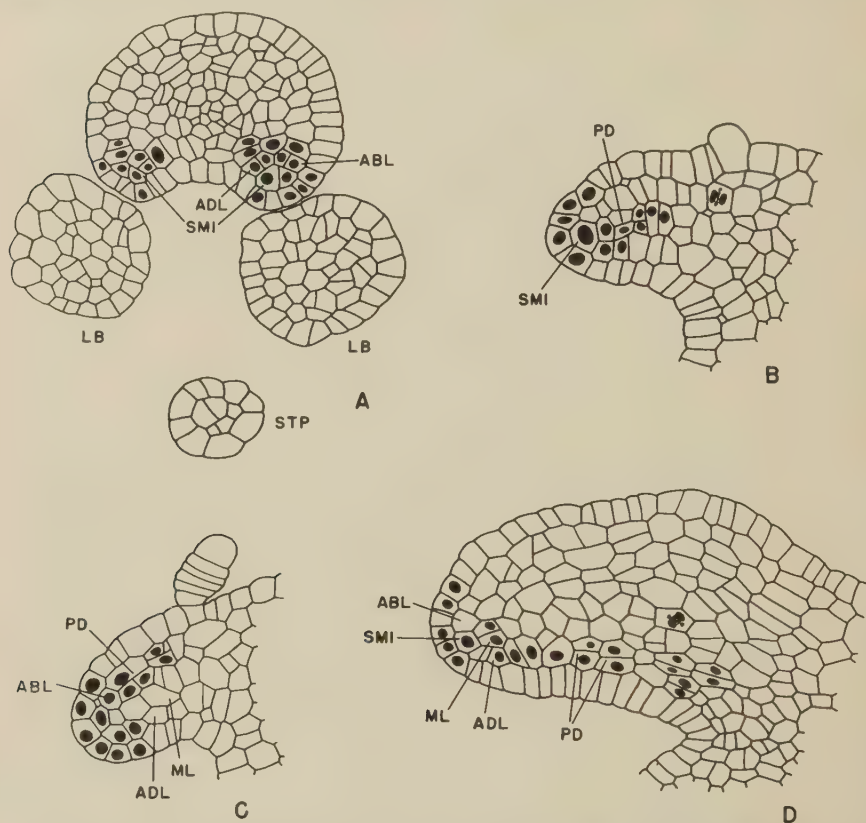


Fig. 8. *A-C*, Portions of foliage leaves from untreated cotton plants showing methods of marginal growth. *D*, Portion of foliage leaf from a treated cotton plant showing early method of marginal growth. Height of primordia and distance from leaf base, respectively (expressed in microns): *A*, 296, 184 (from base); *B*, 1,688, 336 (from base); *C*, 980, 572 (from base); *D*, 408, 180 (from base). *ABL*, Abaxial layer; *ADL*, adaxial layer; *LB*, lateral lobe of future lamina; *ML*, middle layer; *PD*, periclinal division or results of such a division; *SMI*, submarginal initial; *STP*, stipule. ( $\times 300$ .)

visions, grows as a stratum which will give rise ultimately to the palisade layer (fig. 9, *C-D*). Derivatives, occupying the abaxial layer, may divide periclinal very early or divisions may be delayed for some time (fig. 9, *B-D*). The middle layer, growing as a discrete stratum, is interrupted only at intervals by divisions which result in procambial formation (fig. 9, *B-D*). Once the four layers are established they are perpetuated by anticlinal divisions (fig. 10, *A-B*). The parallel layers of cells between developing vascular bundles constitute the so-called "plate meristem" (Schüëpp, 1926). Sub-



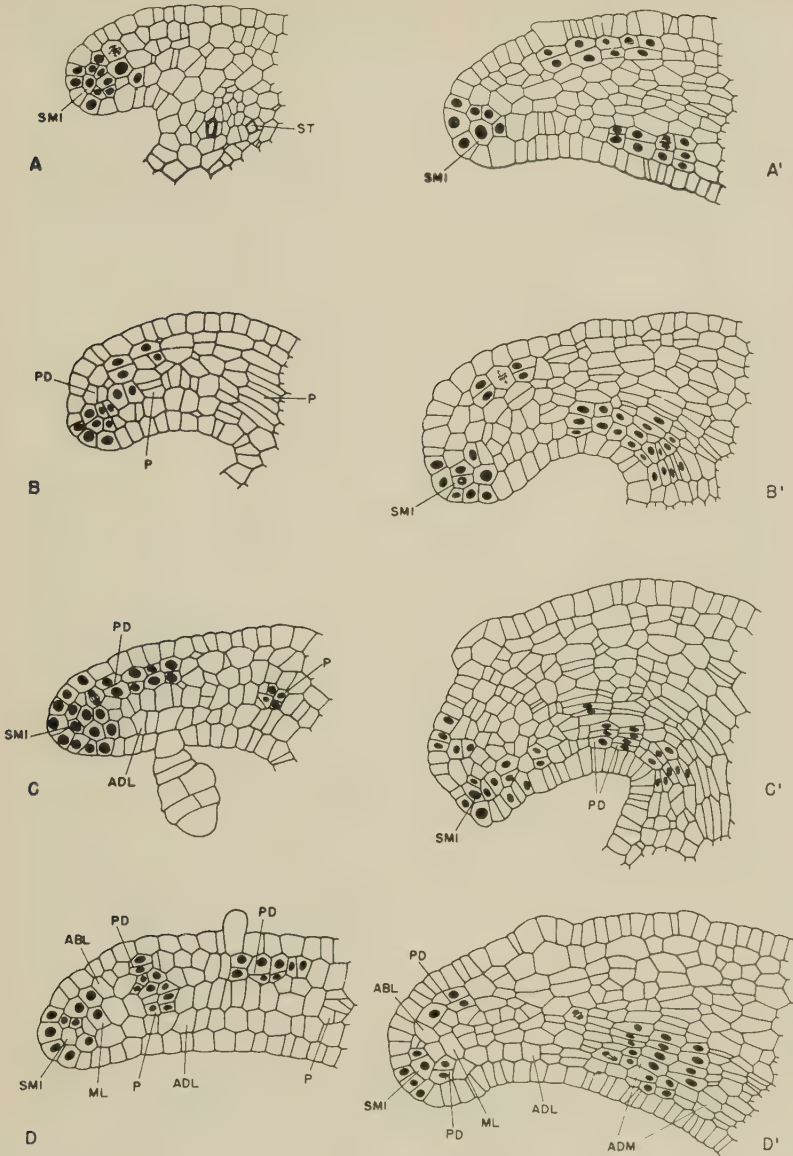


Fig. 9. Comparable levels of foliage leaves in untreated cotton plants (A-D) and cotton plants treated with 2,4-D (A'-D') showing histological details of marginal growth.

Example	Leaf number from apex	Height of leaf, microns	Distance from base, microns	Example	Leaf number from apex	Height of leaf, microns	Distance from base, microns
A.....	4	1,688	792	C.....	5	2,118	904
A'.....	4	1,224	563	C'.....	5	1,560	655
B.....	4	1,688	608	D.....	5	2,118	756
B'.....	4	1,224	379	D'.....	5	1,560	546

Abbreviations same as in Figure 8 with addition of: ADM, adaxial meristem; P, procambium; ST, sieve tube. (X 240.)

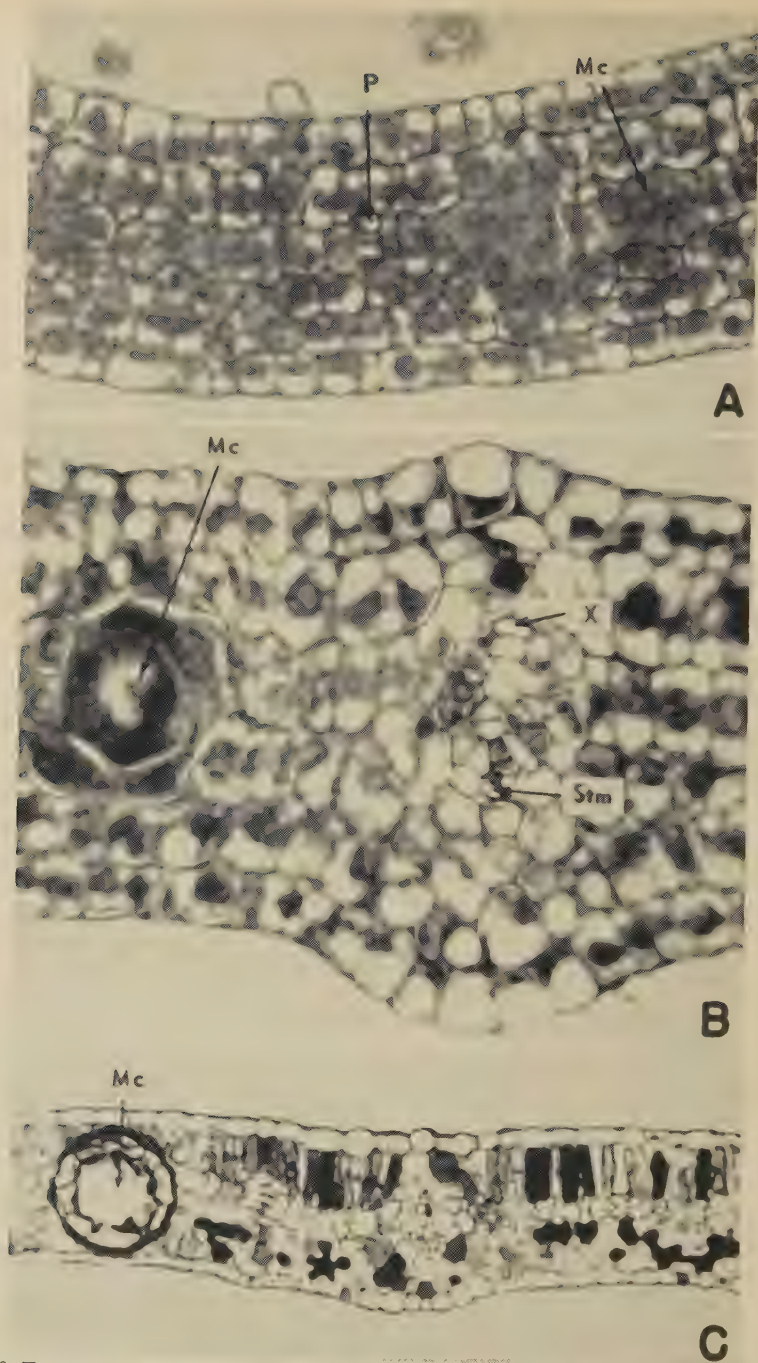


Fig. 10. Transverse sections through foliage leaves of untreated cotton plants. *A*, Foliage leaf in plate meristem stage. Note that the leaf consists of four layers. A minor vein (*P*) is being initiated in derivatives of the original middle layer through pericellular divisions. Early formation of a nascent cavity (*Mc*) is by anticlinal divisions in the middle layer. *B*, Foliage leaf in late plate meristem stage showing a developing lateral vein and a nascent cavity. *Stm*, Stoma; *X*, xylem or tracheary element. *C*, Portion of mature foliage leaf. (*A*,  $\times 340$ ; *B*,  $\times 650$ ; *C*,  $\times 120$ .)

sequent cell enlargement and formation of intercellular spaces causes the over-all expansion of the lamina. The mature leaf has the following mesophyll organization: a palisade layer consisting of one tier of elongate cells in which occasional cells have divided periclinally late in development; a spongy layer composed roughly of three superposed strata derived in part from the original middle layer and in part from early divisions of the abaxial layer (fig. 10, *C*). In the formation of the larger lateral veins all of the original strata participate in the formation of the bundle sheath (fig. 10, *B*).

A convenient method of describing laminal development has been employed by Foster (1936), whereby developmental stages are represented in direct outline form. The type of marginal growth encountered in cotton (var. *Acala*) is illustrated by this method in Figure 11. An examination of this scheme of development reveals a parallel between this type and that reported for several angiosperms. Further consideration will be given to this parallel in the Discussion.

Two aspects of leaf differentiation merit special attention: (1) the formation and development of lateral vascular bundles within the leaf and (2) the development of mucilage cavities.

Lateral vascular bundle formation is initiated by periclinal or oblique divisions in the middle layer of the developing lamina (fig. 9, *C-D*; 10, *A*). Subsequent anticlinal and periclinal divisions increase the thickness of the developing procambial strand. Contributions are made also by the inner derivatives of the abaxial layer, particularly to the phloic part of the procambium (fig. 9, *D*). All veins are collateral in organization, with the phloem located on the abaxial side (fig. 10, *B*), a common arrangement in leaves of seed plants. As mentioned previously, a bundle sheath is formed from cells surrounding the vascular bundle and originates in the ground meristem. In the larger bundles the abaxial and adaxial layers divide periclinally, contributing cells which eventually constitute vein extensions (fig. 10, *B-C*). In the minor veins the entire procambial strand is initiated in, and consists of, derivatives of the middle layer. No conspicuous bundle sheaths or vein extensions are formed.

Lysigenous mucilage cavities are found in the cortex of stem and petiole and in the lamina of foliage leaves. In the lamina of a developing foliage leaf a mucilage cavity originates in the middle layer. In contrast to the early development of veins by periclinal divisions in cells of the middle layer, rapid anticlinal divisions in a cell of the middle layer precede the formation of a mucilage cavity (fig. 10, *A*). While the cells resulting from the first divisions enlarge, with their nuclei becoming greatly distended, other cells in the immediate vicinity divide. First, the inner cells of the group become disorganized and a cavity is formed. By repeated oblique divisions in surrounding cells, new cells are formed centripetally on the periphery of the continually enlarging cavity (fig. 10, *B*). Many of these new cells undergo enlargement and eventual disintegration, their contents becoming part of the cavity substance. In mature leaves, the cavities are spherical and extend from one epidermis to the other (fig. 10, *C*).

**Vascular Organization within the Petiole of Untreated Plants.** A description of the vascular cylinder, its composition, course of bundles, and variations



in organization within the petiole and within the region of transition between petiole and lamina is necessary. This is because late stages in the development of the leaf of treated plants can be explained partially as the continuation of an established developmental pattern at the base of the leaf petiole.

A transverse section near the base of the petiole of a leaf from an untreated plant reveals a cylinder of collateral vascular bundles (fig. 12, *A*). The large abaxial and lateral bundles are extensions of the median and lateral leaf traces, respectively. On the adaxial side is a large bundle (fig. 12, *A*), which at this level represents the fusion of two bundles that are attached to the abaxial bundle at a lower level (fig. 13, *A*). The arrangement of vascular tissues in the nodal region is shown in fig. 13, *A*. Often alternating with these

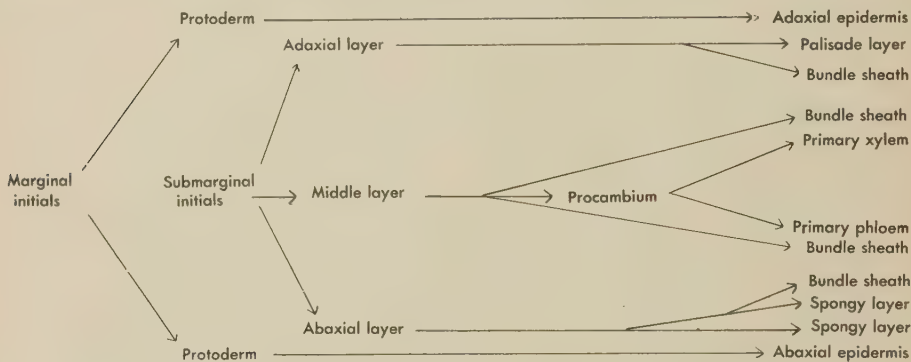


Fig. 11. Outline of marginal growth in the leaf of an untreated cotton plant.

four large bundles are smaller vascular strands (fig. 12, *A-C*), arising from intricate interconnections of the four principal strands within the nodal region.

At approximately midpoint in the mature petiole (fig. 12, *B*), the larger strands are discrete but in the upper part of the petiole the smaller bundles join the four large strands (fig. 12, *C*). Within the last centimeter of the petiole (toward the lamina) vascular strands are present within the original vascular cylinder (fig. 14, *A*). These strands, here called medullary, arise from the sides of the four principal bundles. Often these bundles consist only of phloem, but mostly they are amphivasal in organization. In the region of transition from petiole to lamina these medullary bundles are re-joined to the three basic strands. Furthermore, at this level the large adaxial bundle is dissected into several strands, each one showing a reorientation of the vascular tissues and becoming attached to one of the three remaining vascular bundles (fig. 14, *B*).

**Marginal Growth of Leaves in Treated Plants.** Marginal growth, when established, is fundamentally of the same type as displayed by normal leaves. Submarginal initials divide anticlinally and periclinally giving rise to adaxial and abaxial layers and a middle layer (figs. 8, *D*; 9, *A'-D'*). As in normal leaves, the cells of the abaxial layer soon divide periclinally (fig. 9, *A'-D'*). However, in striking contrast to normal leaves, the treated leaves show pre-

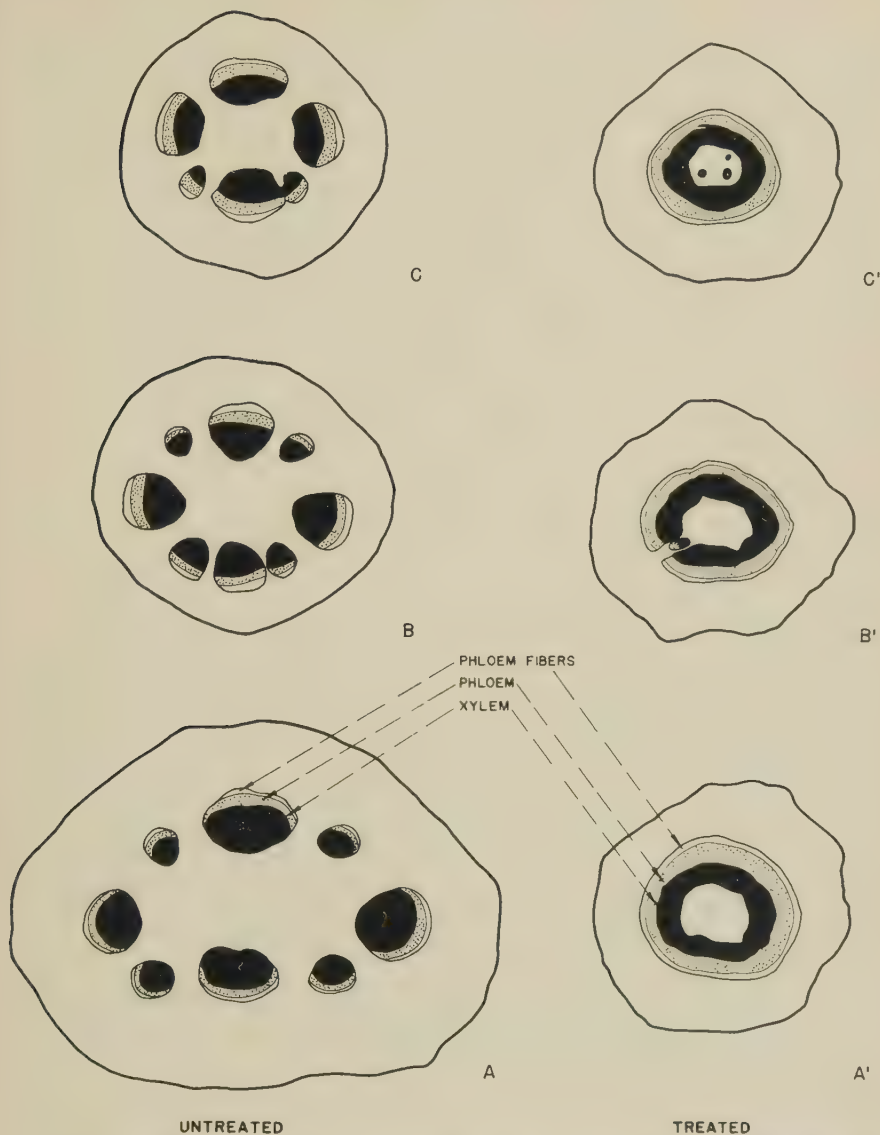


Fig. 12. Comparable levels within the mature petioles of an untreated cotton plant and a cotton plant treated with 2,4-D. *A* to *C* and *A'* to *C'* represent levels from points just above leaf insertion, within the middle region, and upper third, respectively. The adaxial side of the petioles is directed toward the lower edge of the page in all drawings.

cocious periclinal divisions in the cells of the middle layer (figs. 8, *D*; 9, *B'*–*D'*). The immediate derivatives of these divisions continue to divide periclinally as well as anticlinally. While periclinal divisions in the adaxial layer are the exception in the development of the lamina in the normal leaf, such divisions play a significant role in building the future mesophyll of the treated

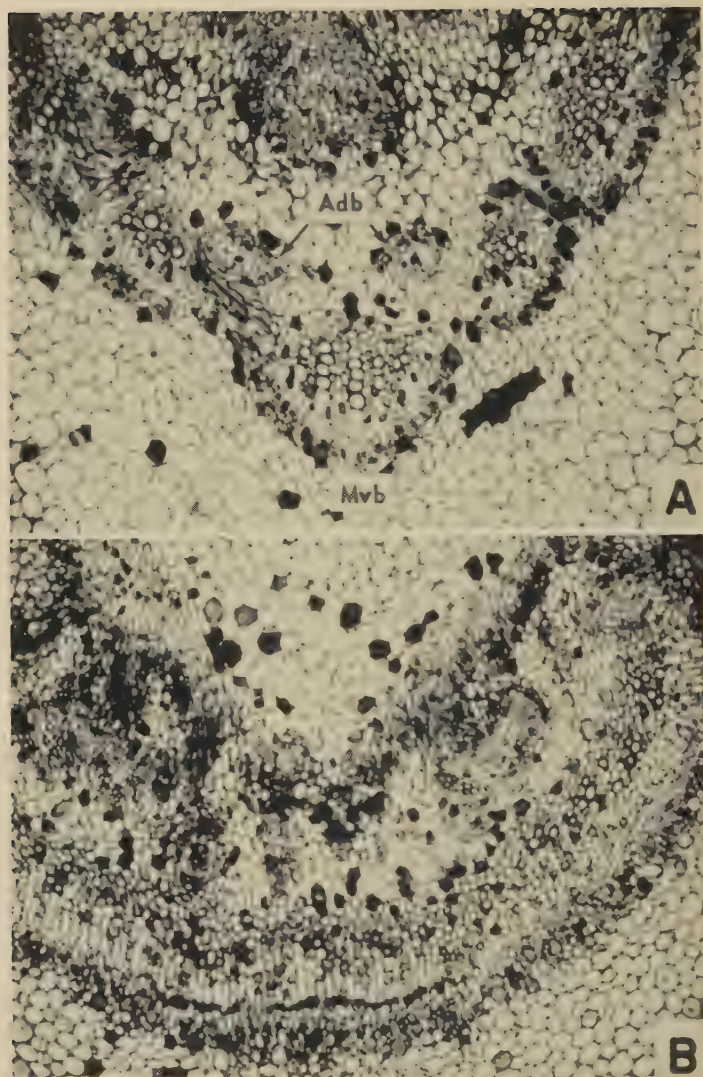


Fig. 13. Transverse stem sections of an untreated cotton plant (*A*) and one treated with 2,4-D (*B*). *A*, The two vascular bundles (*Addb*), branches of the median vascular bundle (*Mvb*) of a foliage leaf are joined at a higher level and constitute the large adaxial bundle seen in Figure 12, *A*. *B*, Most of the vascular complex present at this level merges into a continuous vascular cylinder within the petiole at a higher level. (See fig. 12, *A'*.) (Both  $\times 40$ .)



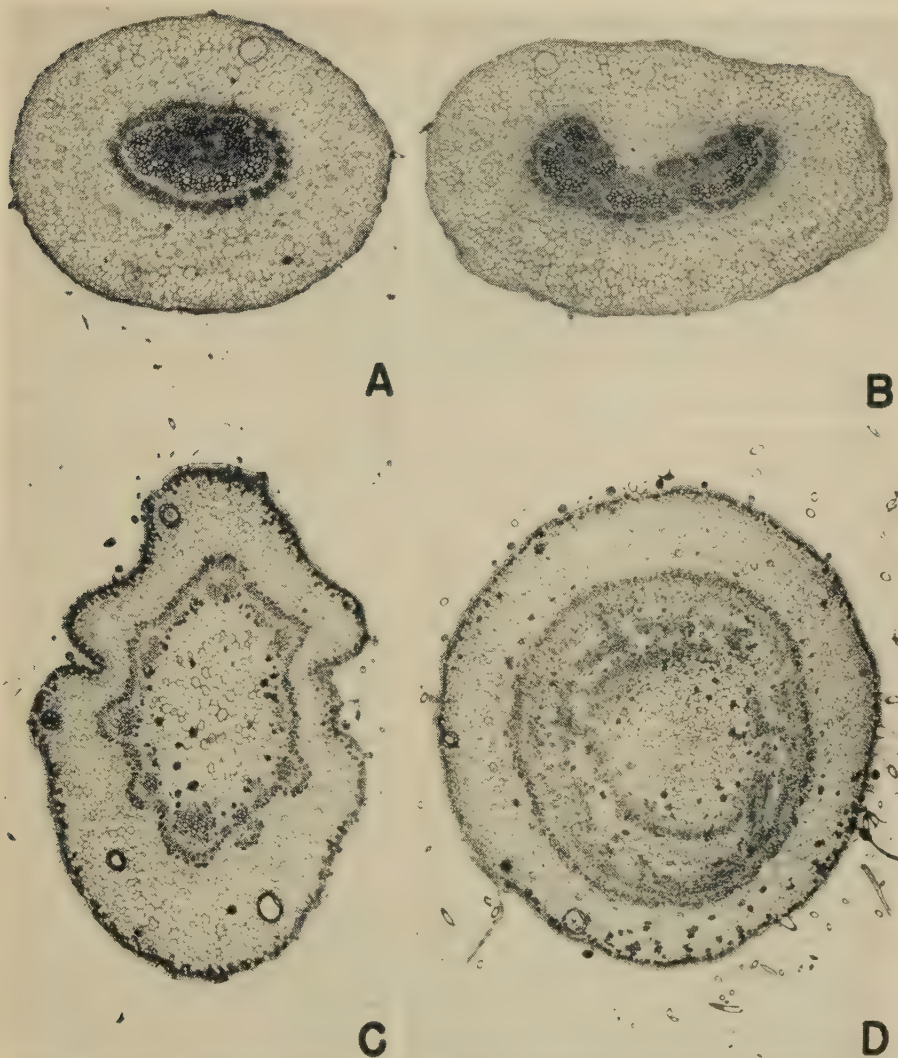


Fig. 14. *A-B*, Transverse sections of a foliage leaf from an untreated cotton plant within the upper one third of the petiole (*A*) and transition region from petiole to lamina (*B*). *A*, At this level the vascular cylinder is continuous except on the adaxial side (toward upper edge of page) where the adaxial vascular tissue is dissected. Medullary bundles are evident within the "pith." *B*, The three basic vascular bundles are evident. The smaller bundles, which, at this level, exhibit reorientation and fusions with the principal bundles, are continuations of the original adaxial bundles and medullary bundles. (Both  $\times 40$ .)

*C-D*, Transverse stem sections of untreated (*C*) and treated (*D*) cotton plants taken at a level 1,029 microns below the shoot apex. A cylinder of leaf traces is visible in *C*. Primary growth is complete in several bundles. In *D*, at this level, the outer continuous ring of vascular tissue represents the fusion product of vascular tissue of at least two ensheathing foliage leaves. An irregular cylinder of leaf traces to younger leaves is present within the outer vascular ring. Interconnections between the two cylinders are apparent on the upper left and lower right of the figure. (Both  $\times 30$ .)

plant. In the leaves of treated plants periclinal divisions are not only characteristic of the adaxial meristem in the midrib region and the adaxial parenchyma in the vicinity of larger veins, but also occur within the interveinal regions. The recognition of divisions in the interveinal regions is difficult in severely affected leaves because the interveinal areas cannot be recognized easily until late in development. The periclinal divisions occur in cells of the adaxial layer only a few cells removed from the submarginal initials (fig. 9,  $C'-D'$ ). By repeated periclinal divisions in the inner and outer cells resulting from the original periclinal divisions within cells of the adaxial layer, a meristematic tissue with oriented divisions is produced (figs. 8,  $D$ ; 9,  $A'-D'$ ). Thus, as the derivatives of the original three layers continue to divide, pre-

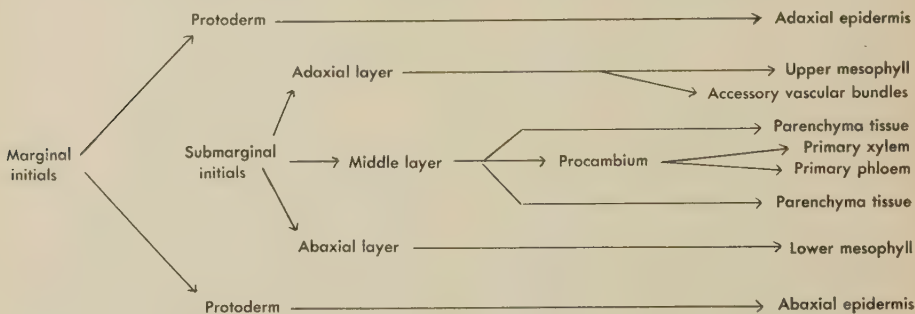


Fig. 15. Outline of marginal growth in the leaf of a treated cotton plant.

dominately in periclinal planes, a massive lamina develops. The phase in development corresponding to the plate-meristem stage is omitted.

The cell lineages which eventually participate in the formation of vascular tissue are difficult to discern. In general, vascular differentiation occurs primarily in derivatives of the middle layer and to some extent in the inner derivatives of the original abaxial layer. In addition to the vascular tissue developed primarily from the derivatives of the original middle layer, additional vascular tissue differentiates rather late in development from the inner layers of cells derived from the original adaxial layer.

Marginal growth in the leaves of treated plants is presented in outline form in Figure 15.

**Vascular Organization within the Petiole in Treated Plants.** Before describing in some detail the differentiation of the additional adaxial vascular strands—here termed accessory vascular bundles—it is necessary to understand vascular organization throughout the petiole in treated plants.

Just above the point of attachment of the mature petiole the vascular tissue forms a continuous cylinder (fig. 12,  $A'$ ). The component bundles are not delimited distinctly. Furthermore, the adaxial portion may not be entirely comparable to that of the petiole in an untreated plant. The study of some specimens has indicated that more numerous vascular bundles are involved (fig. 13,  $B$ ) in the composition of this portion than in normal leaves. The cylindrical organization is maintained throughout most of the petiole, but in the upper one third, separate vascular bundles, originating as branches



Fig. 16. *A-B*, Transverse sections of the petiole of a treated cotton plant corresponding in planes of section to those represented in Figure 14, *A* and *B*, respectively. *A*, Note the continuous cylinder of vascular tissue with some interruptions on the adaxial side. Many medullary bundles are evident. *B*, At this level the wide continuous arc of vascular tissue is apparent. Also numerous accessory bundles are present toward the adaxial side of the leaf. (Both  $\times 40$ .)



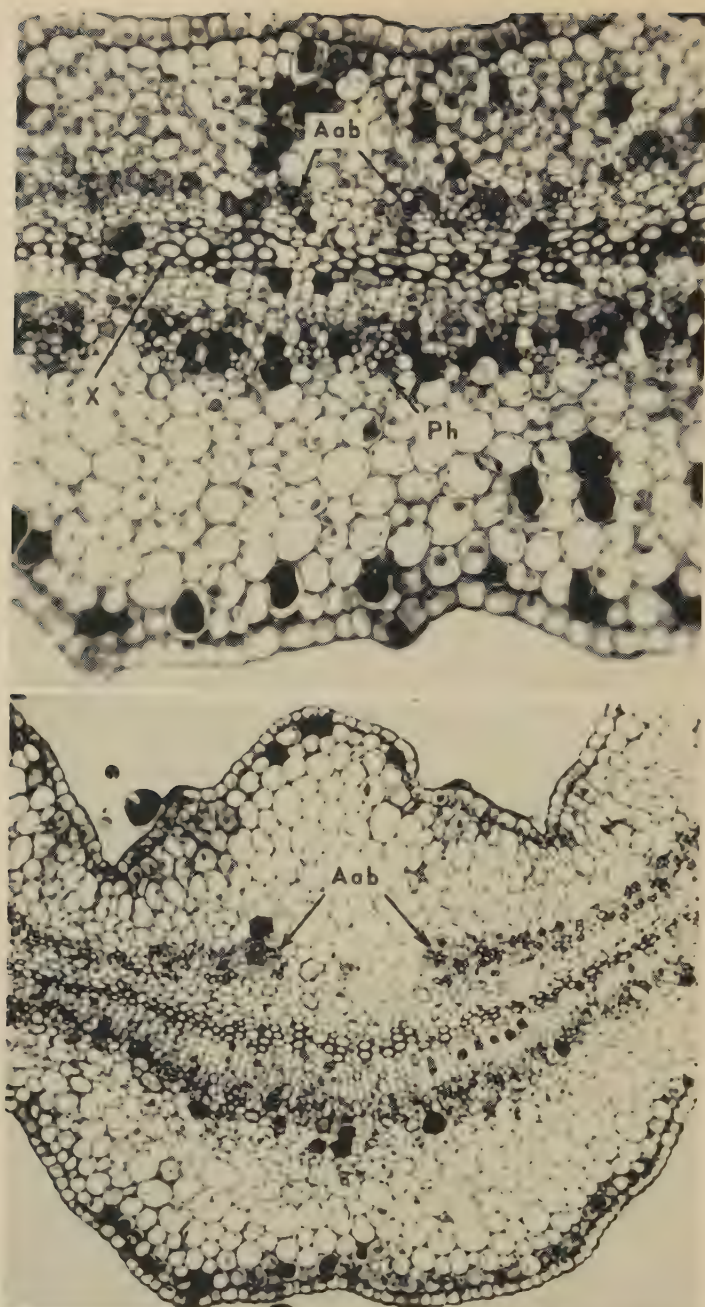


Fig. 17. Transverse sections of foliage leaves in varying stages of development from cotton plants treated with 2,4-D. Above, section through the lamina of a nearly mature foliage leaf showing developing accessory strands, most of which have at least one mature sieve-tube member, not evident at this magnification. Below, mature foliage leaf in the vicinity of the median region of the lamina. A continuous line of vascular tissue as well as the additional adaxial accessory bundles can be identified. *Aab*, Adaxial accessory bundle; *Ph*, phloem; *X*, xylem. (*A*,  $\times 240$ ; *B*,  $\times 120$ .)

from the existing cylinder, diverge into the parenchymatous core, becoming medullary bundles (fig. 12,  $B'-C'$ ). In the upper centimeter of the petiole, depending upon the severity of leaf modification, several to many separate vascular bundles are present in the central core of parenchyma (fig. 12,  $C'$ ). Each of these separate strands is amphivasal in organization. In the region of transition from petiole to lamina the adaxial arc of vascular tissue is dissected (fig. 16,  $A$ ); some of the component parts are joined to the flanks of the now continuous large abaxial arc. The majority of the medullary strands are continuous along the adaxial side of the lamina, becoming aligned horizontally (adaxial to the primary xylem of the "abaxial vascular system," figs. 16,  $B$ ; 17,  $A-B$ ; 22,  $A$ ). In less severely affected plants, the xylem incompletely surrounds the phloem, the two often approaching a collateral arrangement with the phloem being directed toward the adaxial side of the lamina.

## EARLY VASCULARIZATION OF THE SHOOT IN TREATED AND UNTREATED PLANTS

**The Longitudinal Course of Development of Xylem and Phloem.** During this investigation it became apparent that the differentiation of primary xylem and phloem in cotton is greatly affected by the amount of 2,4-D that actually becomes effective. As was mentioned, the leaves in both untreated and treated plants are initiated at approximately the same rate. However, subsequent rates of growth of primordia vary considerably, not only between comparable primordia of control and treated plants, but also between comparable primordia of similarly treated plants.

The direction of initial differentiation of xylem and phloem is essentially the same in untreated and treated cotton plants. Differentiation of sieve tubes within the phloem is acropetal and in continuity with existing differentiated elements. The first xylem elements differentiate in close proximity to the leaf axil, subsequent differentiation being acropetal into the leaf and basipetal until continuity of xylem is achieved at a lower level within the axis. This direction of differentiation is similar to that in the vegetative shoots of many seed plants (Esau, 1943).

Treated and untreated plants differ in the time of appearance and number of differentiated tracheary and sieve elements present at a given level. Figures 18 and 19 illustrate schematically, vascular differentiation within comparable leaves from three separate plants.

In each example of the untreated plant, the three basic leaf traces (median and two laterals) and their extensions into the leaf are shown. The point of leaf insertion (level 0) and the level at which branches from the lateral vascular bundles enter the stipules are indicated. In Figure 18,  $C$ , one sieve tube has become differentiated well into the developing leaf. Isolated series of tracheary elements are also in evidence.

During early leaf development in treated plants it is extremely difficult to distinguish the median and lateral vascular bundles; instead there appear to be numerous small procambial bundles arranged in an arc (fig. 18,  $D$ ). This structural arrangement may be the result of a subdivision of the three leaf traces, or of an addition of strands originating from the vascular tissue pres-

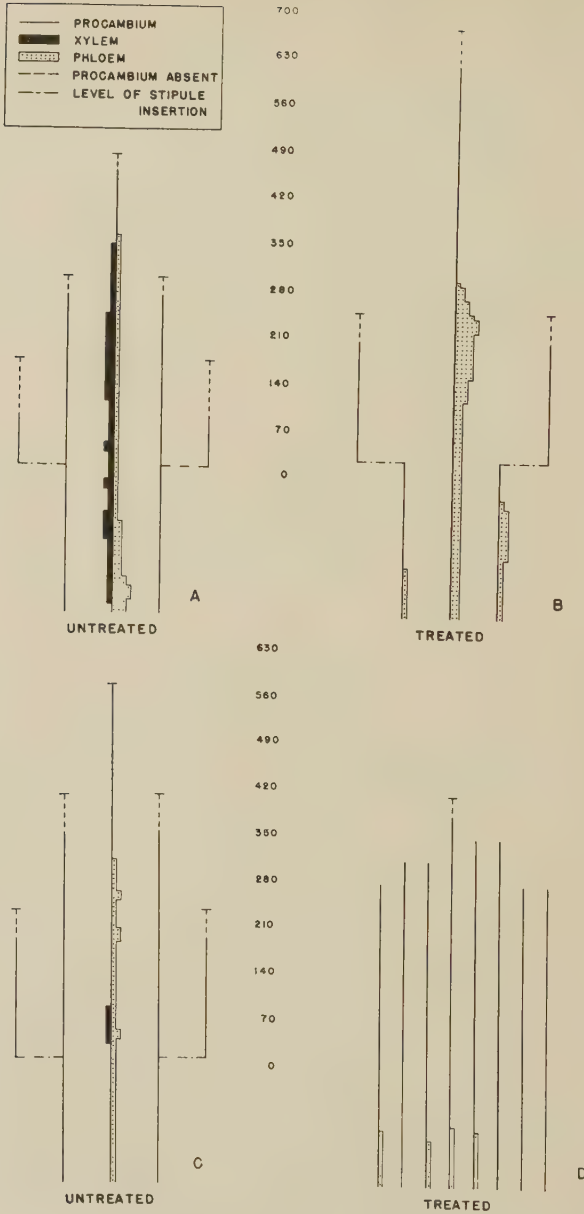


Fig. 18. Schematic representation of the development of primary xylem and phloem in the foliage leaves of untreated cotton plants and plants treated with 2,4-D. Each pair, A-B and C-D, represents the third foliage leaf from the shoot apex in plants of the same age. See text for complete explanation.



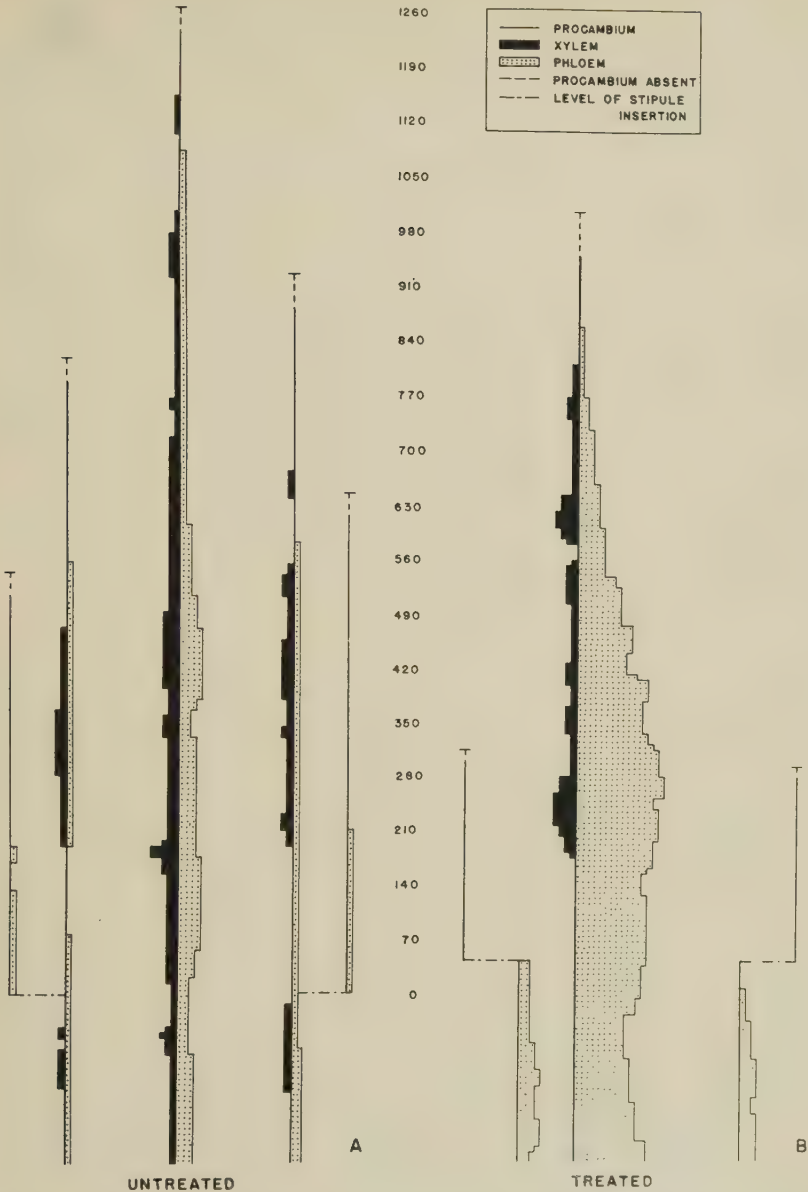
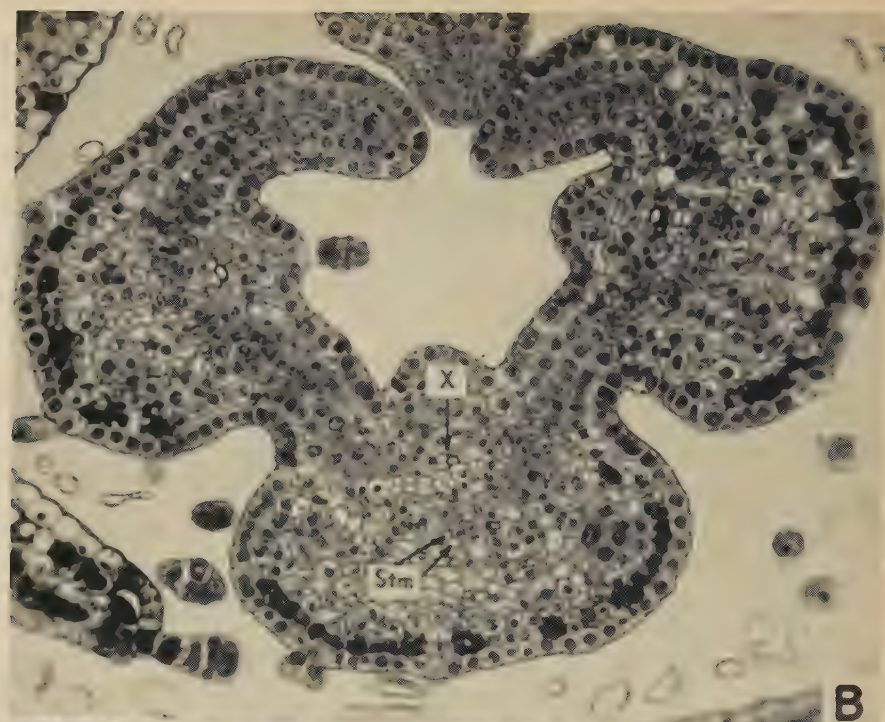
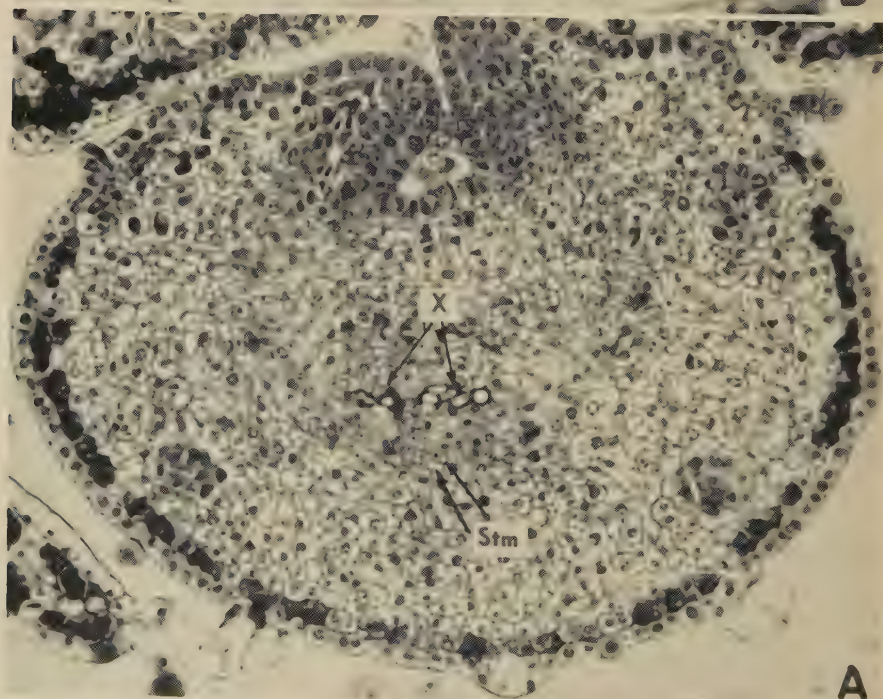


Fig. 19. Schematic representation of the development of primary xylem and phloem in the fourth foliage leaf (from the apex) of an untreated cotton plant and a cotton plant treated with 2,4-D. See text for complete explanation.



**B**



**A**

Fig. 20. Two levels within the fourth foliage leaf (below the shoot apex) from an untreated cotton plant. Height of leaf, 1,520 microns. Levels above leaf insertion, in microns, A, 240; B, 480. *Stm*, Sieve tube member; X, groups of xylem or tracheary elements. (A,  $\times 240$ ; B,  $\times 260$ .)

ent within the shoot at the level of leaf insertion. Both of these processes appear to be operative (note fig. 13, *B*). The possibility that the basic vascular pattern within the shoot has been disturbed and that additional vascular tissue diverges into the leaves, should not be excluded until the vascularization in treated and untreated plants is thoroughly investigated. In this connection compare the sections of the stems of untreated and treated cotton plants at comparable levels (fig. 14, *C-D*).

Figure 18, *A*, shows the median and lateral leaf bundles and the bundles of the stipules. To simplify the diagrams depicting vascular development in the leaves of treated plants in the other comparable pairs (figs. 18, *A-B*; 19, *A-B*), all differentiated vascular tissue located above the level of stipule attachment in these leaves, has been shown as one strand (fig. 19, *B*). If *A* and *B* of Figure 18 are compared, it will be noted that the leaf from the treated plant is considerably taller than the control leaf. It has a greater number of differentiated sieve tubes, yet lacks mature xylem elements. The corresponding leaf from the untreated plant (fig. 18, *A*) has relatively more numerous differentiated tracheary elements than sieve tubes, particularly above the point of leaf insertion. In the two older leaves (fig. 19, *A-B*) it will be noted that an unbroken series of tracheary elements is present (within the region shown in the diagram) in the normal leaf, while in the leaf of the treated plant the differentiated xylem elements are present only some distance above leaf insertion. A rapid increase in the total number of sieve tubes is a characteristic developmental feature of leaves 4 and 5 (below the apex) in treated plants.

To illustrate more completely the differences described above, transverse sections of comparable leaves at representative levels are shown in Figures 20, *A-B*; 21, *A-B*; and 22, *B*. At the region of transition between petiole and lamina, several differentiated tracheary elements and two mature sieve-tube members are visible in the median bundle of a leaf of an untreated plant (fig. 20, *A*). At a comparable level in a treated plant, no tracheary elements are present, but many mature sieve-tube members can be identified on the periphery of the wide arc of differentiating vascular tissue (figs. 21, *A*; 22, *B*). Radiating files of cells (the adaxial meristem) are conspicuous toward the adaxial side of the primordium. It is within this region that differentiation of the accessory bundles occurs.

At a higher level within the same leaf of an untreated plant (fig. 20, *B*), the three principal veins are evident with at least one differentiated tracheary element and sieve-tube member present in each bundle. In the treated leaf at a comparable level (fig. 21, *B*), three groups of xylem elements, each consisting of at least one differentiated tracheary element, are present along the adaxial edge of the vascular arc. Magnification in this figure is not sufficient to reveal the presence of approximately 10 differentiated sieve-tube members.

**Transverse Course of Differentiation of Xylem and Phloem.** The early developmental stages of procambium within the stem of untreated plants are exceedingly difficult to follow in detail. The procambial cells divide longitudinally, initially in various planes (fig. 23, *A*), but later primarily in the tangential plane (periclinal division), so that radial rows of cells result (fig.



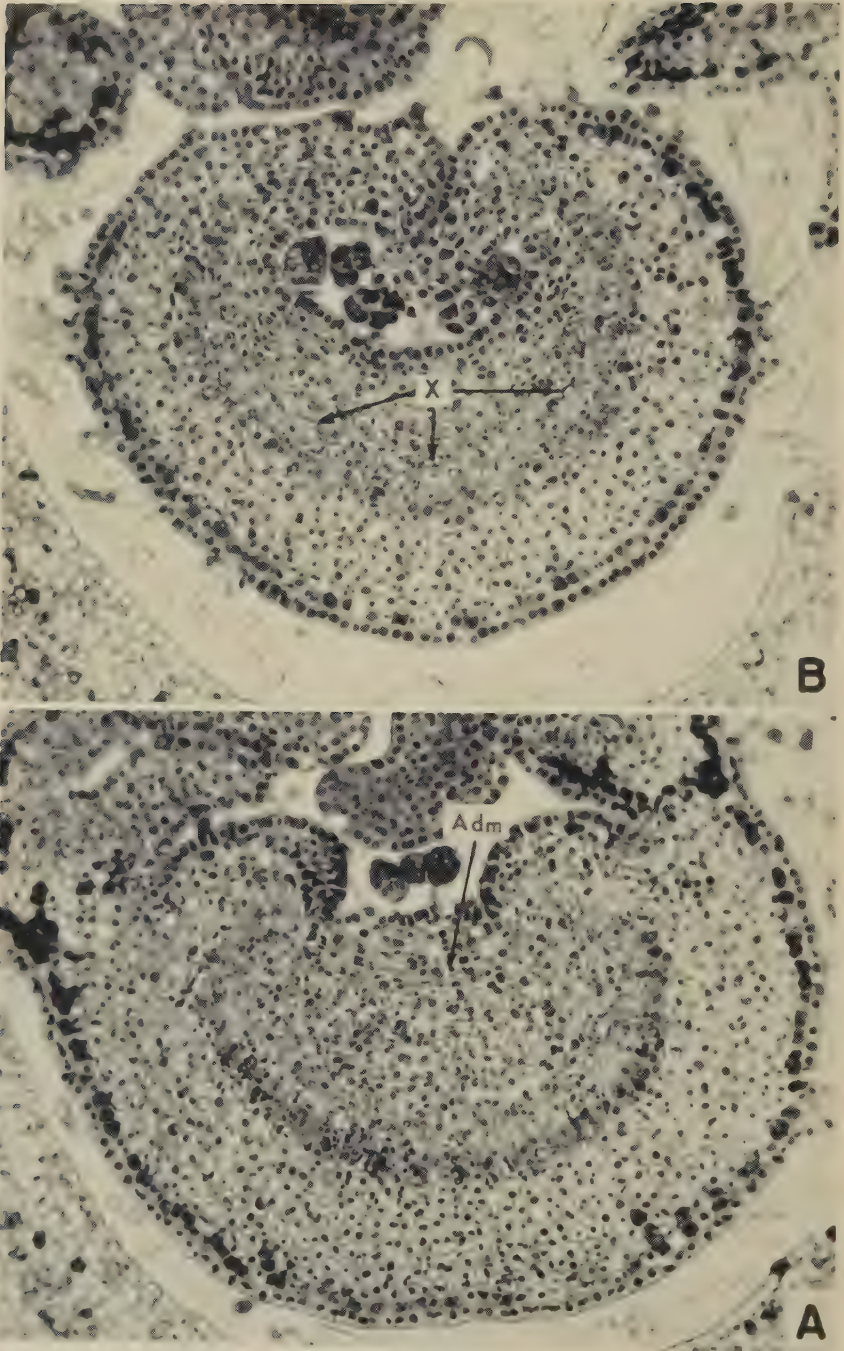


Fig. 21. *A-B*, Transverse sections of a foliage leaf (fourth below the apex) from a treated cotton plant comparable in level to those sections represented in Figure 20, *A* and *B*, respectively. Height of leaf, 1,575 microns. *Adm*, Adaxial meristem; *X*, xylem or tracheary element. (*A*,  $\times 160$ ; *B*,  $\times 170$ .)

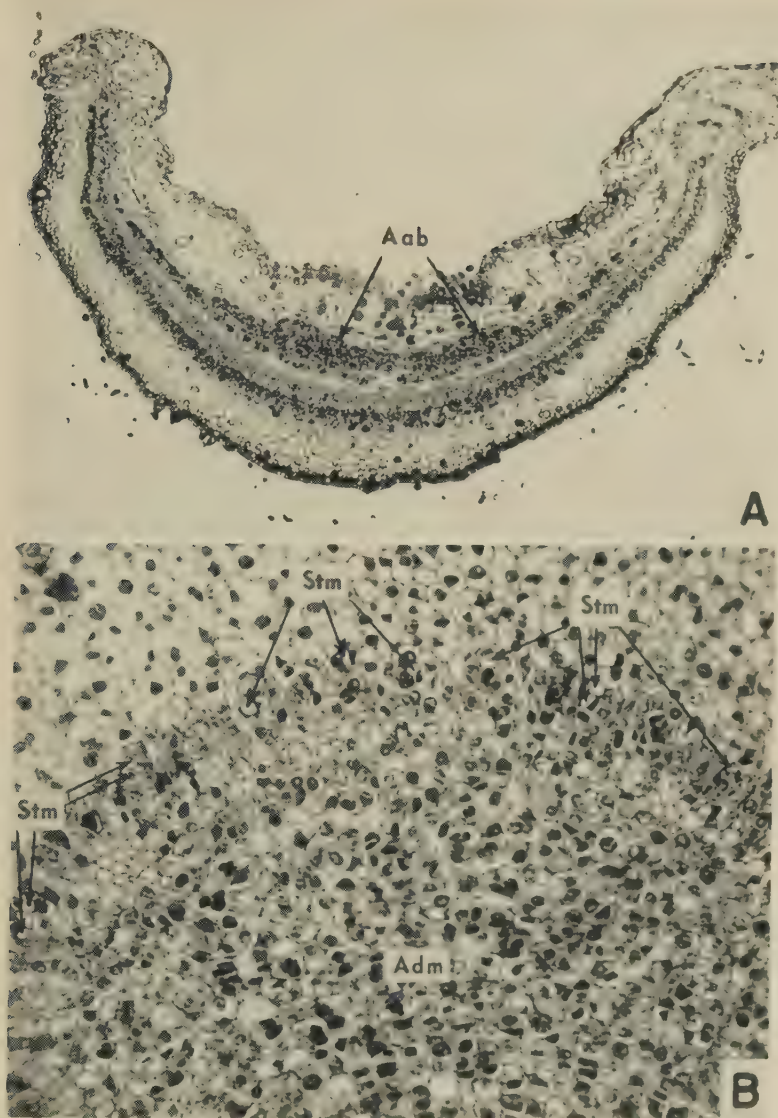


Fig. 22. *A*, Transverse section through a mature foliage leaf of a cotton plant treated with 2,4 D. Note continuous arc of xylem and phloem toward the abaxial side (toward bottom of page) and the presence of a large number of adaxial accessory bundles. *B*, Portion of the developing vascular tissue represented in Figure 21. *A*, shown at a higher magnification. The clear appearing cells at the outer periphery of the arc are mature sieve-tube members. Note absence of mature xylem elements. *Aab*, adaxial accessory bundles; *Adm*, adaxial meristem; *Stm*, sieve-tube member. (*A*,  $\times 30$ ; *B*,  $\times 300$ .)



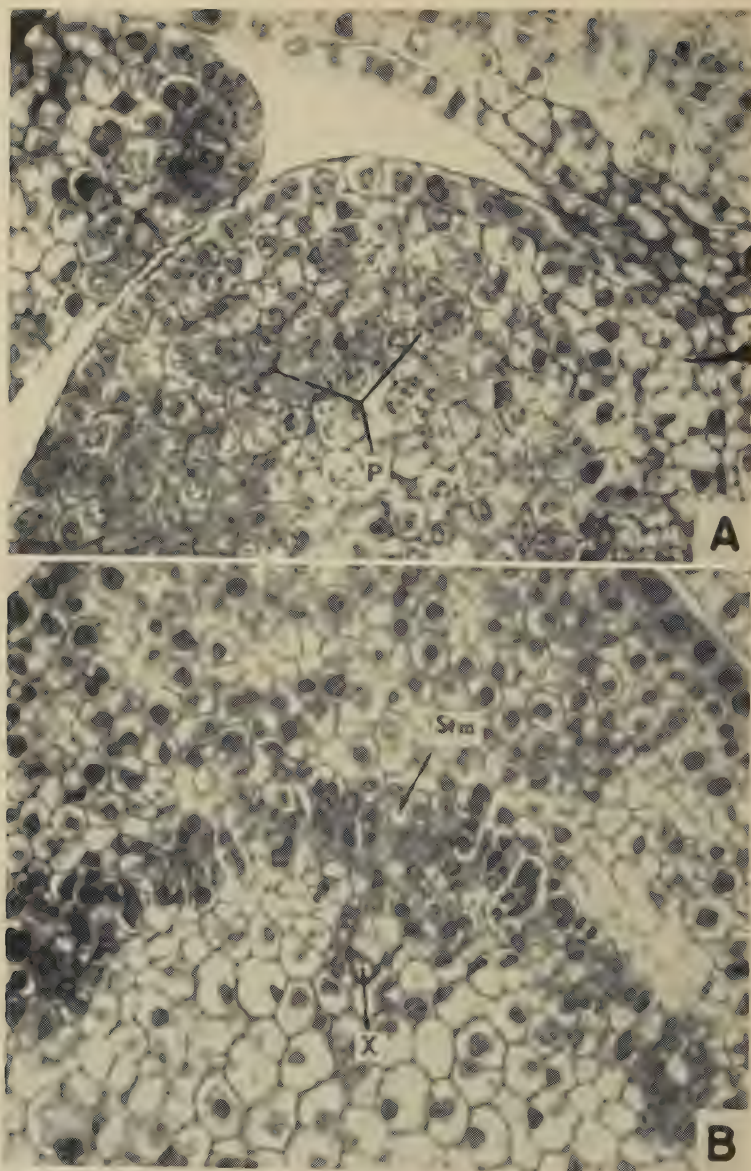


Fig. 23. Transverse sections of stems taken from untreated cotton plants showing stages in the differentiation of procambium (*A*) and of primary vascular tissue (*B*). The levels of section in *A* and *B* are respectively 70 and 176 microns below foliole/leaf insertions; the leaves which these strands are associated with at a higher level are 120 and 760 microns high, respectively. *P*, Procambium; *Sm*, sieve-tube member; *X*, xylem or tracheary element. (Both  $\times 450$ .)



23, *B*). This is particularly true within the central portion of a developing bundle. Along the lateral portions of the bundle, especially within the future phloem regions (phloic procambium), divisions are not oriented in such an orderly fashion (fig. 24, *A*). Procambial cells which will differentiate into tracheary elements of the primary xylem are large, quite vacuolate, and arranged in definite radial rows (figs. 23, *B*; 24, *A*). In Figure 24, *B*, one mature protophloem sieve-tube member and one immature xylem element are present within the differentiating bundle. While additional sieve-tube members become differentiated (fig. 24, *A*), other procambial cells (xylary procambium) continue to divide longitudinally, particularly in the future primary-xylem portion (fig. 24, *A*). Most of these divisions are periclinal, as mentioned above.

A leaf trace, in which secondary growth has just begun, is illustrated in Figure 24, *B*. The metaphloem forms more or less distinct strands, each strand consisting of sieve tubes, companion cells, and parenchyma. Obliterated protophloem sieve tubes can be seen at the periphery of each strand. The tracheary elements of the metaxylem are arranged in definite radial rows.

Early procambial formation is equally difficult to follow in the treated plant. After early establishment, the procambial bundle is increased in width primarily by periclinal divisions (fig. 25, *A-B*). The two separated procambial strands seen in Figure 25, *A*, enter the same leaf. Often two widely separated sieve tubes differentiate at the periphery of the procambium (fig. 25, *B*). In this instance, a protophloem sieve-tube member has differentiated within each of the two distinct procambial bundles. As described earlier, xylem differentiation is delayed; rather late in leaf-trace development, the first xylem element appears some distance from the first sieve-tube member (fig. 26, *A*). In this instance all of the vascular tissue visible in the photograph constitutes the median leaf trace of a leaf at a higher level. After the first xylem element appears, the surrounding vacuolate cells undergo longitudinal divisions in various planes, augmenting the cells of the future primary xylem. The uniform radial alignment of cells, as seen in the leaf trace of the control plant, is not achieved. Differentiation of xylem elements is centrifugal but arrangement into radial rows is obscure (fig. 26, *B*). In Figure 26, *B*, obliteration of considerable protophloem is readily apparent, being replaced by metaphloem. This example has been chosen to show the limits of the bundle. In more severely affected plants the boundaries of all leaf traces are exceedingly difficult to determine.

## DISCUSSION

**Movement of 2,4-D in the Plant.** The application of 2,4-D in solution to the foliage leaves (or cotyledons in the present investigation) poses the question of how the substance enters the leaf and is transported to other parts of the plant, where its effect becomes apparent externally by such responses as curvature of shoot, leaf modification, stunting, and formation of tumorlike outgrowths. Crafts (1951) has emphasized the importance of 2,4-D as a translocation tracer. 2,4-D, when applied to a leaf surface (particularly during periods of high photosynthetic activity or immediately after exposure to light), enters the leaf through the outer tangential walls of

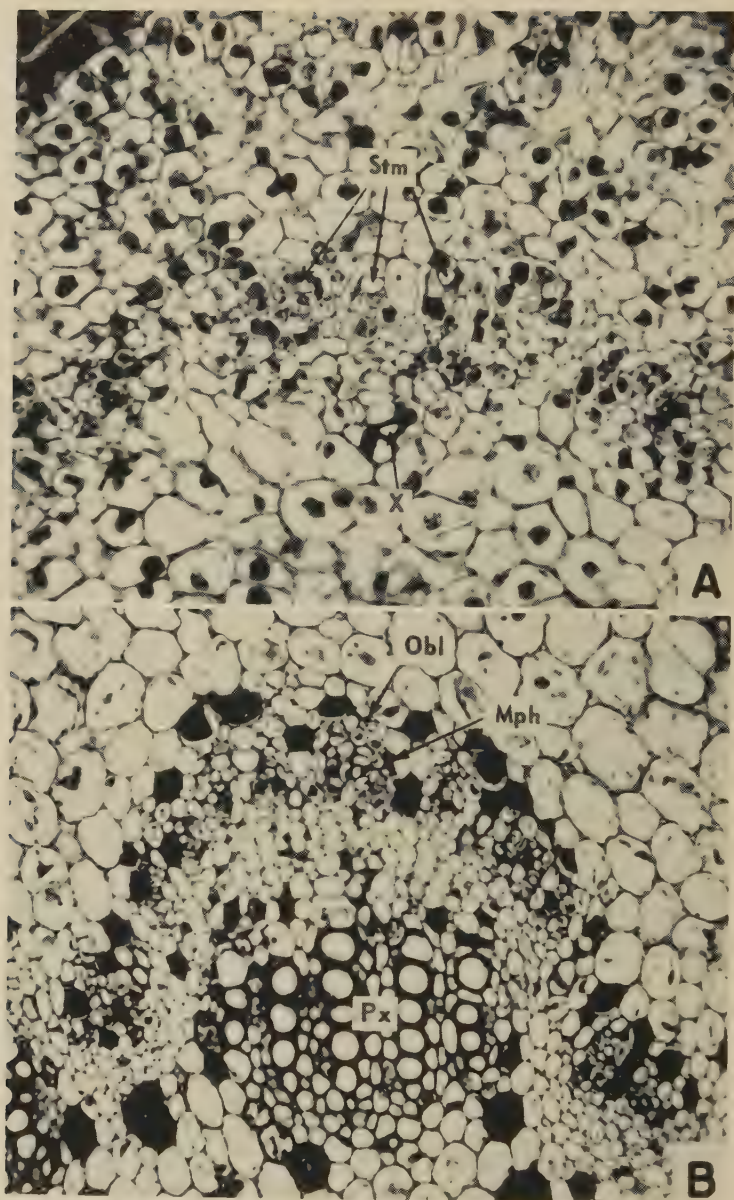


Fig. 24. Transverse sections of stems taken from untreated cotton plants showing stages in the differentiation of vascular tissue. The levels of section in *A* and *B* are respectively 176 and 560 microns below leaf insertion. These vascular strands are the leaf traces of leaves which are 1,416 and 2,300 microns high, respectively. *Stm*, Sieve-tube member; *X*, xylem or tracheary element; *Obl*, obliterated sieve tubes; *Mph*, metaphloem, *Px*, primary xylem. (*A*,  $\times 450$ ; *B*,  $\times 250$ .)

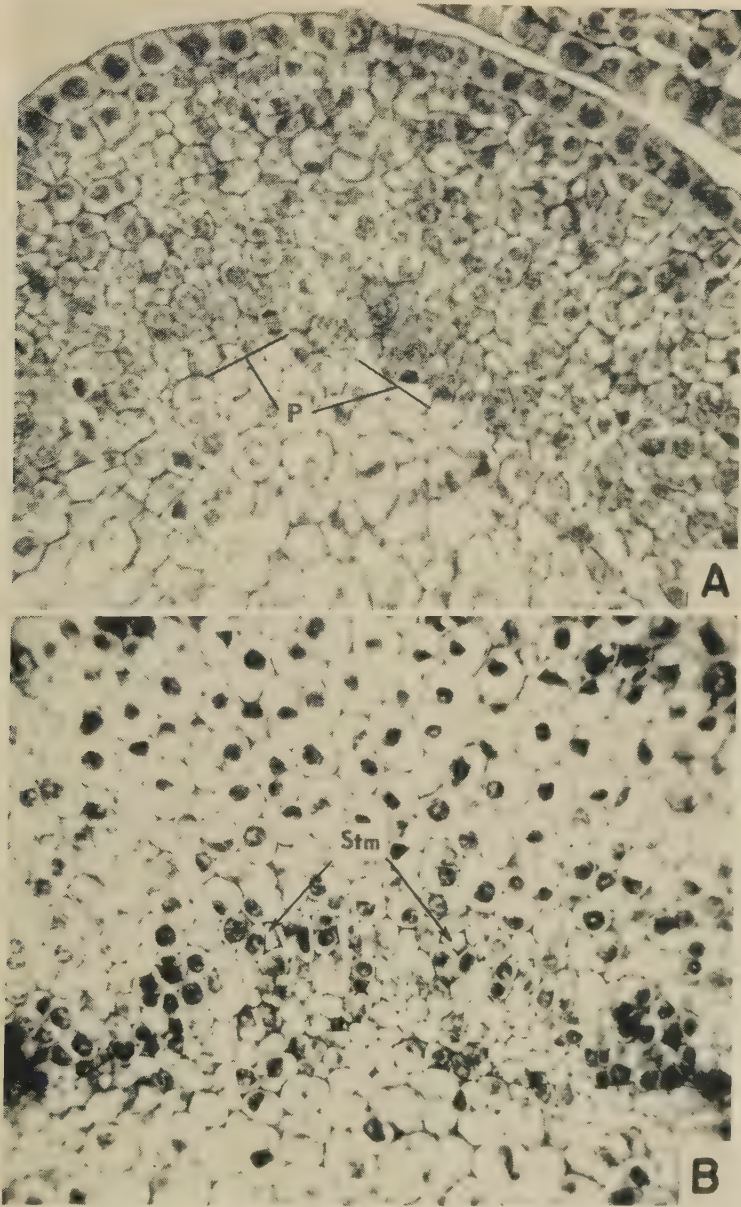


Fig. 25. Transverse sections of stems taken from cotton plants treated with 2,4-D showing stages in the differentiation of procambium (*A*) and of primary vascular tissue (*B*). The levels of section in *A* and *B* are respectively 70 and 105 microns below leaf insertion. These strands are associated with leaves which are 371 and 609 microns high, respectively. *P*, Procambium; *Stm*, sieve-tube member. (Both  $\times 450$ .)



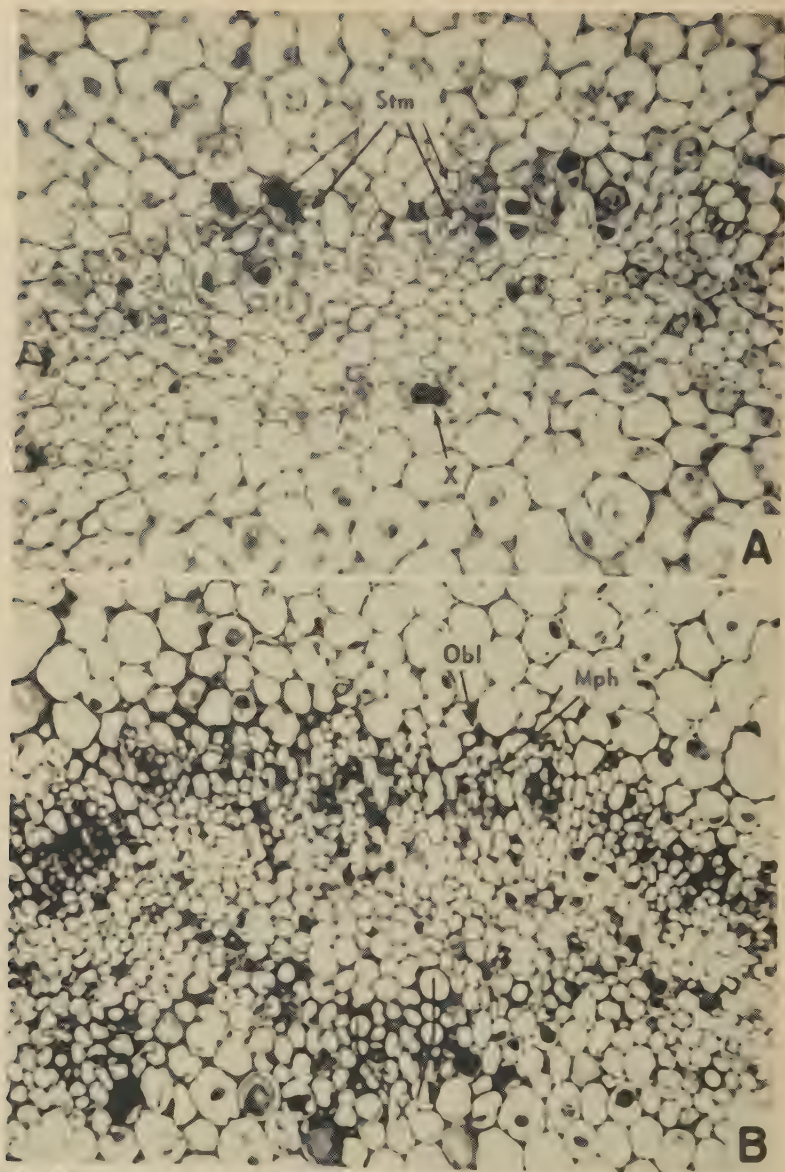


Fig. 26. Transverse sections of stems from cotton plants treated with 2,4-D showing stages in the differentiation of vascular tissue. The levels of section in *A* and *B* are respectively 196 and 1,204 microns below leaf insertion. These bundles are associated with leaves which are 2,005 and 3,000 microns high, respectively. *Stm*, Differentiated sieve-tube member; *X*, xylem or tracheary element; *Mph*, metaphloem; *Obl*, obliterated protophloem sieve tubes. (*A*,  $\times 300$ ; *B*,  $\times 200$ .)

epidermal cells, migrates through the mesophyll, and is moved rapidly with foods in the phloem, causing responses in the stem at a lower level. Of particular interest is the work of Day (1950), in which it was determined that in young bean plants absorption at leaf surface and movement to the phloem through the mesophyll requires about an hour; translocation through the leaf and petiole to the stem requires about 10 minutes. It can be assumed that in cotton a similar sequence of events takes place since the methods of treatment were similar. After the passage of 2,4-D from the cotyledons to the young stem, further movement probably occurs directly to the terminal bud where morphological and histological changes result.

**2,4-D Injury and the Stage of Leaf Differentiation.** Watson (1948) found in bean plants that the amount of injury to leaves depends upon their position and degree of differentiation in the bud at the time of treatment. If the bud is treated early, the first (oldest) leaf shows considerable modification; if it is treated later, the first foliage leaf appears to be normal. The degree of modification in successive leaves follows a unimodal curve, first increasing, then decreasing. In beans, the time of greatest vulnerability to treatment, according to Watson, is during the late stage of plate-meristem development or early cellular differentiation.

In cotton the severity of injury followed a similar pattern: moderate on the first foliage leaf, increasing on the next leaf or next few leaves, then decreasing. Though all the plants were treated at the cotyledon stage, the number of leaves showing successively greater modification varied; hence no attempt was made to relate degree of injury to a definite stage of leaf differentiation.

**Persistence of 2,4-D Action in Plant Tissues.** Several workers have reported the general persistence of 2,4-D stimulus within the plant body for some time after treatment (Dunlap, 1948; Tullis and Davis, 1950).

On the other hand, in the opinion of Eames (1949<sup>b</sup>; see also Tukey, 1950), 2,4-D has a rapid and brief action affecting only the existing primordia. This is in agreement with the results of Watson (1948) on beans; he reports that leaves not present in the bud at the time of treatment were not affected. As Eames points out, in plants that form overwintering buds, the effects of 2,4-D that become apparent when shoot expansion occurs in the spring after treatment reflect injury that occurred in buds during the summer or fall of the previous year.

To test for the persistence of 2,4-D action in plant tissues, McIlrath, Ergle, and Dunlap (1951) treated certain plants at the floral primordia stage with 2,4-D. Seedlings grown from seeds produced in bolls that were initiated 8 weeks after treatment exhibited 2,4-D injury. Thus it would appear that 2,4-D was absorbed and translocated to regions of high meristematic activity, with continuing action on primordia which appeared during development of the embryo. Upon germination of the seed, these malformations became apparent.

The results of the present investigation tend to support the view that 2,4-D action may persist for some time: in cotton, a number of leaves which were not initiated in the bud at the time of treatment showed 2,4-D symptoms.<sup>a</sup>

<sup>a</sup> Professor A. J. Eames has indicated to the author in a personal communication that tests on cotton showed it to be much more sensitive to 2,4-D than other crop plants are.

**Shoot Apical Meristem.** Observations on the cellular organization of the apical meristem in treated and untreated plants throw some light on the question of whether the effects of 2,4-D are due to an alteration in the shoot apex or to modified development from a morphologically unaltered apex.

In both treated and untreated cotton plants the shoot apical meristem consists of a two-layered tunica enclosing the corpus. Superimposed upon these two growth zones is a zonal pattern of cytohistological differentiation. Though the shoot apex in treated plants is narrower and higher than in untreated ones, there seem to be no structural differences. Hence the modifications brought about by 2,4-D must occur, not in the apical meristem, but in tissues derived from it.

**Procambial Development.** There is considerable controversy about the time and direction of procambial development within the shoot of seed plants. Certain workers (see Ball, 1949, for review) have described the appearance of a discontinuous procambial strand at the base of a new leaf, with subsequent basipetal development until continuity with existing procambium is established. An opposite view visualizes the acropetal development of continuous procambium and, in some plants, the formation of procambium before the appearance of a new leaf (see reviews by Esau, 1942, 1943; Ball, 1949).

In cotton the early divisions establishing the growth center of the new leaf and the appearance of procambium seem to be concurrent; and the development of procambium is acropetal and continuous.

**Comparative Modes of Marginal Leaf Growth.** The early establishment of three layers of cells in the lamina of the foliage leaf has been described for several angiosperms, notably *Bougainvillea*, *Pelargonium*, *Plectranthus*, *Ligustrum*, *Nicotiana*, and *Kalanchoë* (see review by Foster, 1936), *Viburnum rufidulum* (Cross, 1937), *Artemisia tridentata* (Diettert, 1938), and *Galinsoga parviflora* (Lawalrée, 1948). Subsequently, however, these three layers vary in their contribution to the developing lamina. Of the genera listed, laminal formation in *Bougainvillea* approaches most closely that displayed by cotton, in that the abaxial layer accounts for the bulk of the lower mesophyll, and the middle layer forms procambium.

In untreated cotton plants the future lamina originates by divisions of submarginal initials, located beneath the protoderm, into three basic layers of cells—adaxial, middle, and abaxial. The cells of the adaxial layer differentiate into a single palisade layer. Those of the middle layer grow as a stratum, interrupted at intervals by periclinal divisions which result in procambial formation. Those of the abaxial layer divide periclinally, contributing cells to the lower mesophyll. Lysigenous mucilage cavities, originating by periclinal divisions in cells of the middle layer, show a scattered distribution in the mature foliage leaf.

**Effect of 2,4-D on Marginal Leaf Growth.** In cotton plants treated with 2,4-D the future lamina originates, as in untreated plants, by the division of submarginal initials into three layers of cells; but subsequent growth of the layers is modified by the treatment. Cells in all three layers divide precociously and predominately in the periclinal plane. Divisions of this type continue in derivatives of the adaxial layer of cells until the future laminal region possesses an adaxial meristem similar to that present in the midrib



region of the lamina in an untreated plant. Accessory vascular bundles differentiate in the tissue produced by this meristem. The mature lamina in a severely affected leaf is thick and consists of an adaxial epidermis, a broad zone of adaxial parenchyma tissue containing accessory vascular bundles, a plate of laterally contiguous abaxial vascular bundles, a zone of abaxial parenchyma tissue, and an abaxial epidermis.

The tissue in severely affected leaves that is composed of relatively unspecialized, isodiametric, vacuolate parenchyma cells with few chloroplasts and that occupies the regions of normal mesophyll has been referred to as a "replacement tissue" (Eames, 1949*a, b*).

**Physiological Factors Affecting Leaf Morphogenesis.** The modified leaf in treated plants may be considered in the light of a physiological concept held by Went (1951). On the basis of evidence from genetical experimentation, teratological expressions, and the responses of leaves to light, viruses, and growth substances, he postulates that there are essentially two groups of factors that control leaf morphogenesis. The first, the caulocaline group, controls stem growth and vein development within the leaf. The second, the phyllocaline group, controls mesophyll development. If this contention be proved correct, the highly modified cotton leaf formed after treatment of the plant with 2,4-D suggests that perhaps this phytocide may be interfering directly or indirectly with the action of phyllocaline, but may have a stimulating effect on the action of caulocaline.

**Proliferative Tissue and the Course of Differentiation of Vascular Elements in the Shoot of Treated Plants.** Considerable information has been accumulated in recent years on the histological responses of roots and stems to growth-regulating substances (Beal, 1945, 1946; Tukey, Hamner, and Imhofe, 1945; Murray and Whiting, 1946, 1947; Swanson, 1946; Whiting and Murray, 1946, 1948; Eames, 1949*a*, 1950).

Much of the work was performed by applying the growth-regulating substance to exposed portions of decapitated stems, although some information is available concerning the effects on the intact plant. In general the plants were treated with relatively high concentrations of growth-regulating substances. Responses of the various tissues of the stem are similar irrespective of the kind of growth-regulating substance used.

All of the workers have described the high sensitivity of a layer interpreted by them as endodermis; very early after treatment, cells of this layer undergo divisions. Frequently the so-called pericycle shows some activity; the parenchyma cells of the primary phloem divide at a rapid rate. Parenchyma cells of the interfascicular region divide, keeping pace with those of the fascicular regions. The cambium is particularly active, forming a broad zone of meristematic derivatives in which differentiation of tracheary elements is delayed.

In the present study on cotton, no large amount of proliferative tissue was formed in the stems of treated plants, possibly because the amount of the herbicide used was small. In extreme cases, there is a general increase in size of the stem, hardly attributable to the activity of any specific region of the stem. However, at levels where primary growth is complete, a broad zone of undifferentiated tissue may be present, derived in part from the vascular cambium, in which delay in maturation of secondary xylem elements is par-

ticularly noticeable. In younger portions of the stem, as in foliage leaves, formation of primary xylem is retarded and that of sieve tubes accelerated as compared with untreated plants.

The direction of differentiation of vascular elements is the same as in untreated cotton and many other seed plants: the first xylem element differentiates near the leaf axil, subsequent differentiation is acropetal and basipetal; sieve-tube elements differentiate acropetally and in continuity with those previously formed in the axis.

**Accessory Vascular Bundles.** Several workers report in other plants the occurrence of accessory vascular bundles which differentiate within tissues derived from the endodermis and phloem parenchyma. Notably, the work of Murray and Whiting (1947) on the effect of 2,4-D and four of its salts on the bean plant illustrates this growth response. Within the derivatives of the endodermis and the primary phloem, centrifugal to the large vascular bundles, isolated tracheids with reticulate thickenings become differentiated; additional complete vascular bundles also become organized within the derivatives. Similar responses were noted in decapitated bean plants treated with tryptophane and low concentrations of indoleacetic acid (Murray and Whiting, 1946). After the latter treatment it was found that in some plants additional vascular bundles became organized from derivatives of the endodermis and the primary phloem parenchyma, and may extend some distance in the modified stem before becoming attached at a lower level to secondary tissue of the vascular cylinder. Similarly, vascular bundles were differentiated from meristematic cells of the vascular cambium and its immature derivatives in decapitated bean plants treated with nicotine (Whiting and Murray, 1946).

The intact cotton plant treated with a low concentration of 2,4-D shows a similar response, in that additional vascular bundles differentiate at nodal regions. The exact ontogeny of these additional strands has not been investigated, but in a sense they are accessory (fig. 13, *B*), although at a higher level they become part of the vascular cylinder of the petiole. At certain levels of the stem in many plants, the cylinder of leaf traces is surrounded by a continuous cylinder of vascular tissue which represents the fusion of vascular bundles of ensheathing leaves. The formation of numerous medullary bundles in the petiole and accessory bundles in the lamina (figs. 12, *C'*; 16, *A*; 17, *A-B*; 22, *A*) has already been discussed.

**Effects of 2,4-D on the Sieve-Tube Elements of the Phloem.** Eames (1950) followed in detail the fate of primary phloem in the hypocotyl of bean plants sprayed with 125 p.p.m. of 2,4-D. All tissues between the cortex and primary xylem were involved in the production of a characteristic meristematic proliferation. In the primary phloem, which was mature at the time of treatment, the phloem-parenchyma cells underwent active cell division, becoming part of the meristematic tissue. Within this activated meristem the sieve tubes of the primary phloem became disrupted and displaced and the smaller sieve tubes and companion cells were crushed. After 8 to 13 days, only remnants of the primary phloem were visible and no secondary phloem sieve tubes differentiated within the proliferated region up to the last day of the experiment. The conclusion was drawn that phloem destruction is a factor in bringing about the death of plants treated with 2,4-D.

In contrast, there is an increase in the number of differentiated sieve tubes in the young leaf and stem of cotton plants treated with nonlethal dosages of 2,4-D. The treated cotton plants of this study exhibit histological alterations that would seem to permit ready movement of available food materials into the young developing shoot. The amount of translocatable photosynthate must, however, be limited by the altered adult structure of leaves. Whether there are subsequent radical changes in the phloem of more mature regions of the stem has not been investigated.

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